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IX. Mitteilung

Einfluss verschiedener Chemikalien auf die Funktion des RES und der Leber

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Seit die Chemotherapie von Ehrlich[1] aufgestellt worden ist, sind durch die raschen Fortschritte der Chemie und Pharmazie verschiedene Chemikalien hergestellt worden. Aber der Wirkungsmechanismus solcher Chemikalien im lebenden Körper ist noch nicht ganz klar. Wie schon aus der VIII. Mitteilung[2] dieser Arbeit ersichtlich ist, steigern z.B. Sulfonamide und Antibiotika die Funktion des RES.

Deshalb haben wir vorliegende Untersuchungen unternommen, um zu erfahren, welchen Einfluss verschiedene Chemikalien auf die Funktion des RES und der Leber ausüben. Die Auswahl der Versuchstiere sowie die Methodik war die gleiche wie bei der früheren Mitteilung[3].

Versuchsteil

Versuchsergebnisse

1. Einfluss verschiedener Eiweisspräparate

Wie man aus Tabelle I und II ersieht, vermindert sich der Kongorotindex und bleibt die Prothrombinzahl unverändert durch die Injektion von Eryosan (3% Kasein) in Menge von 0.2 ccm pro kg. Aber die Injektion von Jecuron (Gewebseiweisspräparat), Mari-Amin (Rinderblutpräparat), Vita-X (Placentarpräparat), Neo-Soteira (Globulinpräparat) oder Neo-Minophagen AT (Lysinpräparat) verursacht eine Verminderung des Kongorotindex und eine Vermehrung der Prothrombinzahl, mit Ausnahme von Jecuron. Im Gegensatz dazu ruft die Injektion von Paniltin (Cysteinpräparat) in Menge von 1 ccm pro kg oder Histamin

TABELLE I

Einfluss verschiedener Eiweisspräparate

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Eryosan	3 % 0.2 ccm s.c.	K-index	1	80.4	59.2	76.3	64.3
			2	70.2	71.4	80.0	68.4
			Durchschn.	74.5	65.0	78.2	66.3
			Differenz		−20.3	−15.4	−25.7
		P-zahl	1	1.00	1.00	0.96	1.03
			2	1.00	1.00	1.00	0.97
			Durchschn.	1.00	1.00	0.98	1.00
			Differenz		0.01	0.01	0.02
Jecuron	1 ccm s.c.	K-index	1	48.8	53.2	59.7	47.4
			2	45.4	58.8	66.7	50.0
			Durchschn.	46.9	56.2	63.2	48.8
			Differenz		−2.5	−2.8	−15.6
		P-zahl	1	1.00	0.97	0.98	1.03
			2	1.00	0.97	0.96	0.95
			Durchschn.	1.00	0.97	0.97	0.99
			Differenz		−0.02	0	0.01
Mari-Amin	5 % 1 ccm i.v.	K-index	1	62.9	65.7	70.0	67.2
			2	55.5	76.8	72.2	69.5
			Durchschn.	58.9	71.6	73.0	68.5
			Differenz		0.9	−5.0	−7.1
		P-zahl	1	1.00	0.98	1.01	1.10
			2	1.00	0.99	1.00	1.04
			Durchschn.	1.00	0.99	1.01	1.07
			Differenz		0	0.04	0.09
Vita-X	1 ccm s.c.	K-index	1	62.5	61.7	65.2	65.0
			2	52.3	65.0	65.2	61.7
			Durchschn.	57.7	63.2	65·2	63.3
			Differenz		−6.3	−11.8	−11.8
		P-zahl	1	1.00	0.99	1.04	0.95
			2	1.00	1.03	0.99	0.99
			Durchschn.	1.00	1.01	1.02	0.97
			Differenz		0.02	0.05	−0.01
Neo-Soteira	1 EH s.c.	K-index	1	57.6	64.0	71.0	67.8
			2	54.0	64.7	56.2	71.6
			Durchschn.	55.5	64.4	63.0	71.8
			Differenz		−0.9	−11.6	−1.2
		P-zahl	1	1.00	1.05	1.06	0.99
			2	1.00	1.03	1.06	1.01
			Durchschn.	1.00	1.04	1.06	1.00
			Differenz		0.05	0.09	0.02

in Menge von 0.1 mg pro kg eine Vermehrung des Kongorotindex, aber Histamin in Menge von 0.03 mg pro kg seine Verminderung hervor, während die Prothrombinzahl in jedem Falle unverändert bleibt.

TABELLE II

Einfluss verschiedener Aminosaurer Präparate

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Neo-Minophagen AT	1 ccm s.c.	K-index	1	60.5	62.5	52.5	66.7
			2	50.2	57.0	53.2	53.7
			Durchschn.	55.6	59.8	52.8	61.5
			Differenz		−7.6	−21.9	−11.6
		P-zahl	1	1.00	1.02	0.98	1.11
			2	1.00	1.09	0.98	0.98
			Durchschn.	1.00	1.06	0.98	1.05
			Differenz		0.07	0	0.07
Paniltin	1 ccm s.c.	K-index	1	54.4	58.0	72.7	70.7
			2	48.0	50.0	60.0	76.4
			Durchschn.	51.6	54.2	66.2	73.3
			Differenz		−0.6	10.3	18.0
		P-zahl	1	1.00	0.99	1.09	1.09
			2	1.00	1.01	0.95	1.00
			Durchschn.	1.00	1.00	1.02	1.05
			Differenz		−0.01	−0.01	0.02
Histamin	0.1 mg i.m.	K-index	1	43.0	71.3	73.2	71.5
			2	40.9	53.8	70.0	46.3
			Durchschn.	42.2	63.8	68.2	58.4
			Differenz		18.4	21.7	12.5
		P-zahl	1	1.00	0.93	1.03	1.05
			2	1.00	1.00	1.01	1.03
			Durchschn.	1.00	0.97	1.02	1.04
			Differenz		−0.04	−0.01	0.01
	0.03 mg i.m.	K-index	1	43.6	45.0	45.8	52.7
			2	41.0	38.5	43.7	43.1
			Durchschn.	42.4	41.9	44.8	68.4
			Differenz		−3.7	−2.9	2.3
		P-zahl	1	1.00	0.98	1.03	1.03
			2	1.00	1.00	1.01	0.99
			Durchschn.	1.00	0.98	1.02	1.01
			Differenz		−0.03	−0.01	−0.02

Daraus wird klar, dass diese Eiweisspräparate, nicht aber Paniltin, die Funktion des RES steigern, dagegen diejenige der Leberzellen nur durch Mari-Amin, Vita-X, Neo-Minophagen AT und Neo-Soteira erhöht wird.

2. Einfluss verschiedener Anti-asthmatischer Mittel

Wenn man Benadrin (Benadryl) in Menge von 30 mg pro kg oder Anergen in Menge von 10 mg pro kg unter die Haut injiziert, so entsteht, wie in Tabelle III gezeigt wird, eine Verminderung des Kongorotindex mit nachheriger Vermehrung, während die Prothrombinzahl zur Verminderung neigt. Weiterhin vermindert sich der Kongorotindex durch

TABELLE III

Einfluss verschiedener Anti-asthmatischer Mittel

	Dose (pro kg)		Nr. des Kaninchen	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Benadrin	30 mg s.c.	K-index	1	38.6	42.9	59.2	56.7
			2	34.3	38.4	67.8	60.0
			Durchschn.	36.8	41.0	62.8	58.0
			Differenz		−9.6	13.3	11.0
		P-zahl	1	1.00	1.04	1.06	1.00
			2	1.00	1.00	1.00	1.01
			Durchschn.	1.00	1.02	1.03	1.00
			Differenz		0.02	0.04	−0.02
Anergen	10 mg s.c.	K-index	1	52.3	66.7	65.2	66.7
			2	50.0	50.0	73.3	84.0
			Durchschn.	51.2	58.3	70.5	74.0
			Differenz		−4.7	0.2	5.3
		P-zahl	1	1.00	1.01	0.93	0.91
			2	1.00	0.97	0.93	0.93
			Durchschn.	1.00	0.99	0.93	0.92
			Differenz		0	−0.04	−0.06
Minophagen C-Stark	1 ccm s.c.	K-index	1	42.9	50.1	59.5	45.8
			2	41.1	55.0	40.0	40.0
			Durchschn.	41.9	52.8	48.3	43.0
			Differenz		−2.9	−6.3	−9.1
		P-zahl	1	1.00	0.98	1.00	1.00
			2	1.00	0.97	0.97	0.93
			Durchschn.	1.00	0.98	0.99	0.97
			Differenz		−0.02	0	−0.05
Neudrin	1 ccm s.c.	K-index	1	53.0	76.0	82.4	84.2
			2	50.0	85.6	55.3	61.6
			Durchschn.	51.6	80.6	69.4	72.8
			Differenz		17.2	−1.3	3.7
		P-zahl	1	1.00	0.92	1.02	0.93
			2	1.00	0.99	0.95	0.96
			Durchschn.	1.00	0.95	0.98	0.94
			Differenz		−0.04	−0.01	−0.06

die Injektion von Minophagen C-Stark (Glycyrrhizinpräparat), im Gegensatz dazu vermehrt er sich durch Neudrin (Methylephedrin), in Menge von 1 ccm pro kg, während die Prothrombinzahl in jedem Falle zur Verminderung neigt.

Daraus ist ersichtlich, dass anti-asthmatische Mittel wie Benadrin, Anergen und Minophagen C-Stark, nicht aber Neudrin, die Funktion des RES steigern, während die Leberzellenfunktion in jedem Falle zur Verminderung neigt.

3. Einfluss verschiedener Anti-ulceröser Mittel

Wie aus Tabelle IV zu ersehen ist, ruft die Injektion von Larostidin

TABELLE IV

Einfluss verschiedener Anti-ulceröser Mittel

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.) 1	3	5
Larostidin	1 ccm i.m.	K-index	1	49.3	44.0	47.3	53.3
			2	46.5	44.7	60.8	64.0
			Durchschn.	48.0	44.4	54.2	58.6
			Differenz		−17.4	−6.5	0.4
		P-zahl	1	1.00	1.00	1.00	0.98
			2	1.00	0.99	0.99	0.99
			Durchschn.	1.00	1.00	1.00	0.99
			Differenz		0	0.01	−0.03
Umor	1 ccm i.v.	K-index	1	54.3	46.7	50.0	66.7
			2	44.4	42.6	42.9	45.8
			Durchschn.	49.2	44.7	46.9	55.9
			Differenz		−18.3	−15.0	−3.5
		P-zahl	1	1.00	0.97	0.95	0.96
			2	1.00	0.97	0.94	0.95
			Durchschn.	1.00	0.97	0.95	0.96
			Differenz		−0.04	−0.08	−0.07
Umor-Stark	1 ccm s.c.	K-index	1	48.2	77.1	67.7	67.7
			2	48.0	85.1	62.0	67.7
			Durchschn.	48.1	81.0	65.2	65.5
			Differenz		21.1	−2.2	−0.1
		P-zahl	1	1.00	1.12	1.14	1.24
			2	1.00	0.92	0.97	0.89
			Durchschn.	1.00	1.02	1.06	1.07
			Differenz		0.03	0.09	0.09
Stimulin M	1 ccm i.v.	K-index	1	69.5	46.9	57.5	57.0
			2	59.2	55.8	57.2	71.3
			Durchschn.	64.4	51.7	57.3	65.0
			Differenz		−9.5	3.5	−1.5
		P-zahl	1	1.00	1.03	1.04	1.04
			2	1.00	1.02	1.03	1.03
			Durchschn.	1.00	1.03	1.04	1.04
			Differenz		0.03	0.03	0.02
Gastamin	1 ccm s.c.	K-index	1	60.1	59.5	45.6	65.5
			2	45.5	44.5	41.2	40.0
			Durchschn.	52.4	52.7	43.7	52.2
			Differenz		−3.2	−13.2	−4.1
		P-zahl	1	1.00	1.07	1.06	1.06
			2	1.00	0.99	1.00	1.05
			Durchschn.	1.00	1.03	1.03	1.06
			Differenz		0.02	0	0.03
Banthine	10 mg i.m.	K-index	1	51.7	63.7	59.3	62.7
			2	49.0	50.0	56.8	59.5
			3	48.5	61.1	54.6	59.5
			Durchschn.	49.7	58.3	56.7	62.3
			Differenz		5.4	2.7	8.9
		P-zahl	1	1.00	1.00	1.03	0.96
			2	1.00	1.02	1.01	1.02
			3	1.00	1.00	0.98	0.94
			Durchschn.	1.00	1.01	1.01	0.97
			Differenz		0	−0.02	−0.06
Finalin	10 mg s.c.	K-index	1	70.4	67.5	69.1	69.8
			2	54.5	50.9	69.5	70.8
			Durchschn.	62.8	58.9	69.5	70.2
			Differenz		−15.7	−12.4	−10.1
		P-zahl	1	1.00	0.99	1.07	1.07
			2	1.00	1.04	0.95	0.93
			Durchschn.	1.00	1.02	1.01	1.00
			Differenz		0.03	0.04	0.02

(Histidinchlorhydrat), Umor (Albuminpräparat), Stimulin M oder Gastamin (Magenschleimhautpräparat) eine Verminderung des Kongorotindex hervor, aber die Prothrombinzahl nimmt in keinem Falle zu, während die Injektion von Umor-Stark (Umor mit Scopolia sowie Bilsenkrautextrakt) eine Vermehrung des Kongorotindex sowie der Prothrombinzahl verursacht.

Bei Anwendung von Blockierungsmitteln der vegetativen Ganglien ruft die Injektion von Banthine eine Zunahme des Kongorotindex und eine Abnahme der Prothrombinzahl hervor, während die Finalininjektion ein entgegengesetztes Resultat zeigt.

Daraus geht hervor, dass die Funktion des RES durch Larostidin, Umor, Stimulin M, Gastamin und Finalin gesteigert, aber durch Umor-Stark und Banthine unterdrückt wird, während die Leberzellenfunktion nur durch Umor-Stark gebessert wird.

4. Einfluss verschiedener Anti-canceröser Mittel

Aus Tabelle V sieht man, dass die Injektion von Nitrogen-Mustard oder dessen Derivate wie Nitromin (Nitrogen-Mustard-N-Oxide), Isoamyl-oder Cyclohexyl-Nitromin eine Verminderung des Kongorotindex veranlasst, im Gegensatz dazu vermehrt sich die Prothrombinzahl durch Isoamyl-oder Cyclohexyl-Nitromin. Weiterhin bemerkt man eine Verminderung des Kongorotindex nur durch Orthobromoparaaminotropolone von anti-canceröser Wirkung, nicht aber durch Paraaminotropolone ohne anti-canceröser Wirkung, während die Prothrombinzahl in beiden Fällen unverändert bleibt.

Daraus ersieht man, dass diese anti-cancerösen Mittel zwar die Funktion des RES steigern, aber die Leberzellenfunktion nur durch Isoamyl-oder Cyclohexyl-Nitromin erhöht wird.

5. Einfluss sonstiger Chemikalien

Von den sonstigen Chemikalien verursacht die Injektion von Lumin (Kryptocyaninpräparat), Hikarutin (Rutin), Spractor (Hyaluronidase), Heparin, Greenpole (Chlorophyllpräparat), Solarson (Arsenpräparat), wie in Tabelle VI gezeigt wird, eine Verminderung des Kongorotindex, während die Prothrombinzahl sich nur durch Greenpole und Solarson vermehrt. Betreffs des Leberextraktes ruft die Injektion von Lebercalcin (Gluthationpräparat), Lebercalcin-Stark (Lebercalcin mit Vitamine B_1 und B_2) oder Campolon eine Verminderung des Kongorotindex hervor, aber die Prothrombinzahl wird nur durch Campolon gesteigert. Weiterhin bemerkt man eine Zunahme des Kongorotindex durch Methiocil (Methylthiouracil) und seine Abnahme durch Curachihormon (Autohormon des Verdauungssystems), während die Prothrombinzahl durch das letztere abnimmt.

Daraus wird klar, dass diese Chemikalien, nicht aber Methiocil, zwar

Tabelle V

Einfluss verschiedener Anti-canceröser Mittel

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Nitrogen-Mustard	0.2 mg i.v.	K-index	1	75.8	82.4	78.6	57.3
			2	59.8	65.3	68.6	69.4
			Durchschn.	68.3	73.6	73.7	62.8
			Differenz		−6.5	−13.7	−23.0
		P-zahl	1	1.00	1.00	0.98	1.00
			2	1.00	0.94	0.95	0.95
			Durchschn.	1.00	0.97	0.97	0.98
			Differenz		−0.02	0	0
Nitromin	50 mg i.v.	K-index	1	52.1	50.0	56.7	51.7
			2	50.7	60.8	77.7	70.6
			Durchschn.	51.3	56.0	65.8	63.7
			Differenz		−6.1	−4.6	−5.1
		P-zahl	1	1.00	1.01	1.04	1.01
			2	1.00	0.97	0.96	0.99
			Durchschn.	1.00	0.99	1.00	1.00
			Differenz		0	0.03	0.02
	5 mg i.v.	K-index	1	63.3	61.8	64.0	60.8
			2	59.6	54.8	63.4	65.8
			Durchschn.	61.7	58.5	63.7	62.8
			Differenz		−15.0	−17.1	−16.4
		P-zahl	1	1.00	1.04	1.01	1.01
			2	1.00	1.01	1.00	0.99
			Durchschn.	1.00	1.03	1.01	1.00
			Differenz		0.04	0.04	0.02
Isoamyl Nitromin	5 mg i.v.	K-index	1	51.1	64.0	61.9	70.0
			2	46.5	54.5	48.0	75.0
			Durchschn.	48.8	59.0	56.4	72.1
			Differenz		−1.6	−11.5	4.8
		P-zahl	1	1.00	1.19	1.10	1.14
			2	1.00	1.12	1.05	1.09
			Durchschn.	1.00	1.16	1.08	1.12
			Differenz		0.17	0.11	0.14
Cyclohexyl-Nitromin	5 mg i.v.	K-index	1	59.0	63.4	72.7	65.2
			2	53.9	69.0	66.6	73.6
			Durchschn.	56.6	66.1	69.9	69.9
			Differenz		−2.3	−5.8	−5.0
		P-zahl	1	1.00	1.00	1.06	1.06
			2	1.00	0.95	1.01	1.06
			Durchschn.	1.00	0.98	1.04	1.06
			Differenz		−0.01	0.07	0·08
Paraamino-tropolone	2 mg s.c.	K-index	1	56.3	64.5	85.3	69.0
			2	55.3	78.1	84.4	64.5
			Durchschn.	57.6	70.6	85.0	67.3
			Differenz		1.2	8.3	−7.8
		P-zahl	1	1.00	1.03	0.94	0.97
			2	1.00	1.01	0.92	0.97
			Durchschn.	1.00	1.02	0.93	0.97
			Differenz		0.03	−0.04	−0.01
Orthobromo-paraamino-tropolone	2 mg s.c.	K-index	1	65.8	63.0	62.5	68.7
			2	61.5	57.0	57.2	60.0
			Durchschn.	63.8	60.3	60.5	65.0
			Differenz		−15.3	−22.4	−16.3
		P-zahl	1	1.00	0.94	1.02	1.01
			2	1.00	0.94	0.94	1.01
			Durchschn.	1.00	0.97	0.98	1.01
			Differenz		−0.02	0.01	0.03

TABELLE VI

Einfluss verschiedener Chemotherapeutika

	Dose (pro kg)		Nr. des Kaninchens	Von Inj.	Zeit nach Injektion (St.) 1	3	5
Lumin	0.2 γ s.c.	K-index	1	58.1	60.1	72.6	74.8
			2	50.0	58.2	57.2	71.0
			Durchschn.	53.3	59.0	63.8	67.0
			Differenz		−6.2	−8.6	−3.8
		P-zahl	1	1.00	1.00	1.00	1.05
			2	1.00	1.00	1.04	1.00
			Durchschn.	1.00	1.00	1.02	1.03
			Differenz		0.01	0.05	0.05
Hikarutin	10 mg s.c.	K-index	1	80.5	61.2	79.3	76.2
			2	45.1	38.3	35.3	35.7
			Durchschn.	60.0	49.9	57.3	57.6
			Differenz		−14.3	−7.0	−6.1
		P-zahl	1	1.00	1.05	1.04	1.00
			2	1.00	1.02	1.02	1.05
			Durchschn.	1.00	1.04	1.03	1.03
			Differenz		0.03	0	0
Spractor	2000 EH i.v.	K-index	1	54.2	50.1	45.0	50.3
			2	53.3	48.7	48.0	54.4
			Durchschn.	53.8	49.4	46.7	52.2
			Differenz		−7.6	−11.4	−5.3
		P-zahl	1	1.00	1.01	1.03	1.04
			2	1.00	0.98	1.02	0.96
			Durchschn.	1.00	1.00	1.03	1.00
			Differenz		−0.01	0	−0.03
Heparin	10 mg i.m.	K-index	1	80.8	43.7	53.7	54.3
			2	74.8	74.3	88.6	51.8
			Durchschn.	78.1	61.5	73.3	52.6
			Differenz		−19.8	−9.1	−29.2
		P-zahl	1	1.00	1.02	1.01	1.04
			2	1.00	1.02	1.02	1.03
			Durchschn.	1.00	1.02	1.02	1.04
			Differenz		0.01	−0.01	0.01
Greenpole	3 mg i.m.	K-index	1	57.3	16.0	54.0	72.8
			2	52.6	53.5	63.8	55.0
			Durchschn.	54.8	60.3	58.8	62.3
			Differenz		−6.3	−15.1	−10.0
		P-zahl	1	1.00	1.00	1.03	1.08
			2	1.00	0.99	0.95	1.06
			Durchschn.	1.00	1.00	0.99	1.07
			Differenz		0.01	0.02	0.09
Solarson	3 mg i.m.	K-index	1	76.5	66.0	50.0	71.0
			2	56.7	62.6	52.5	60.2
			Durchschn.	56.6	66.1	69.9	69.1
			Differenz		−14.6	−34.5	−10.3
		P-zahl	1	1.00	1.05	1.04	1.00
			2	1.00	1.01	1.03	1.02
			Durchschn.	1.00	1.03	1.04	1.01
			Differenz		0.04	0.07	0.03
Lebercalcin	2 ccm s.c.	K-index	1	56.8	57.5	69.0	78.4
			2	46.5	50.0	50.0	64.8
			Durchschn.	52.2	54.0	60.2	72.5
			Differenz		−2.7	−3.0	3.8
		P-zahl	1	1.00	0.94	0.96	0.98
			2	1.00	0.95	0.91	0.93
			Durchschn.	1.00	0.95	0.94	0.96
			Differenz		−0.06	−0.08	−0.08
Lebercalcin-Stark	1 ccm s.c.	K-index	1	61.1	62.5	65.5	75.2
			2	54.2	41.7	52.9	53.0
			Durchschn.	57.6	52.2	59.0	64.0
			Differenz		−17.2	−17.7	−10.9
		P-zahl	1	1.00	1.06	1.01	1.00
			2	1.00	0.92	0.90	0.90
			Durchschn.	1.00	0.99	0.96	0.95
			Differenz		0	−0.01	−0.03

die Funktion des RES steigern, dass aber die Leberzellenfunktion nur durch Greenpole, Solarson und Campolon erhöht wird.

Diskussion

Es ist schon bekannt, dass das RES bei unspezifischer Therapie eine grosse Rolle spielt, etwa wenn sie durch Injektion von Eiweisskörpern, kolloidalen Metallen oder Farbstoffen durchgeführt wird (Saxl[4]). Besonders berichtete Muda[5] über eine Funktionssteigerung des RES und Nakajima[6] sogar über eine gesteigerte phagozytose der Leukozyten durch Eryosan in kleiner Menge. In Bezug auf Eiweisspräparate bemerkte Niwa[7] eine Funktionssteigerung des RES durch Vita-X; Jansco[8] betonte seine Steigerung durch Histamin, doch leugneten Barrow usw.[9] die obige Wirkung durch Histamin oder Antihistamin.

Aus Tabelle VII sieht man, dass verschiedene Aminosäuren der Eiweisspräparate wie Vita-X, Umor, Stimulin M und Gastamin mittelst der Papierchromatographie nachgewiesen werden. An unseren Ver-

Tabelle VII

Einfluss sonstiger Chemotherapeutika

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)					
					3	5	8	12	24	48
Campolon	1 ccm i.m.	K-index	1	40.5	39.0	41.9	45.3	40.5	44.0	50.0
			2	39.7	45.7	49.2	41.6	42.1	48.0	52.3
			Durchschn.	40.1	42.6	46.2	43.2	41.4	46.1	51.1
			Differenz		-4.3	-7.4	-7.7	-6.2	0.8	2.2
		P-zahl	1	1.00	1.02	1.00	1.03	1.18	1.08	1.02
			2	1.00	1.02	1.04	1.04	1.09	0.99	0.95
			Durchschn.	1.00	1.02	1.02	1.04	1.14	1.04	0.99
			Differenz		0.02	0.03	0.03	0.14	0.04	-0.01
Methiocil	5 ccm i.v.	K-index	1	50.0	57.7	68.2	64.3	62.5	55.5	50.0
			2	44.5	48.4	56.7	51.6	52.6	52.8	46.4
			Durchschn.	48.7	52.8	62.3	57.6	60.0	54.0	48.3
			Differenz		2.9	9.2	8.9	12.5	5.2	0.3
		P-zahl	1	1.00	1.00	1.00	1.03	1.03	1.01	1.02
			2	1.00	1.02	1.00	0.99	1.03	1.01	0.91
			Durchschn.	1.00	1.01	1.00	1.01	1.03	1.01	0.97
			Differenz		-0.01	0	-0.04	-0.02	-0.01	0
Curachi-hormon	1 EH s.c.	K-index	1	56.0	47.8	52.7	50.0	42.5	40.5	52.1
			2	44.2	47.8	47.7	43.5	33.9	40.5	40.5
			Durchschn.	50.1	47.8	50.2	46.8	38.2	40.5	45.9
			D.fferenz		-2.4	-4.3	-3.4	-10.7	-9.1	-3.5
		P-zahl	1	1.00	1.07	0.98	0.98	0.95	0.97	0.97
			2	1.00	0.98	0.96	0.94	0.94	0.94	0.93
			Durchschn.	1.00	1.03	0.97	0.96	0.95	0.96	0.95
			Differenz		0.04	-0.03	-0.07	-0.09	-0.07	-0.04

TABELLE VIII

Papierchromatographisch dargestellte Aminosäurearten

Chemotherapeutika	Dose	Glutaminsäure	Valin od. Methionin	Leu-Isoleusin	Asparaginsäure	Serin	Glycin	Alanin	Prolin	Lysin	Asparagin	Thyrosin	Phenylalanin	Histidin	Arginin	Oxyprolin	Cysteinsäure	Threonin
Vita-X	0.1 ccm	++	++	++	++	++	++	++	+	++	−	+	+	+	+	+	−	±
Umor	0.05 ccm	++	++	++	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Umor-Stark	0.1 ccm	++	++	++	++	++	++	++	+	++	−	+	+	+	+	±	−	±
Stimulin M	1 ccm	++	+	+	++	++	±	+	−	−	+	−	−	−	−	−	−	−
Gastamin	0.04 ccm	++	+	##	++	##	±	+	+	−	++	−	−	−	−	−	++	−

suchsergebnissen zeigt sich also, dass verschiedene Eiweisspräparate wie Eryosan, Mari-Amin, Jecuron, Vita-X, Neo-Minophagen AT, Neo-Soteira und Histamin in kleiner Menge die Funktion des RES steigern, aber Paniltin und Histamin in grosser Menge seine Funktion unterdrücken. Betreffs verschiedener anti-asthmatischer Mittel beobachteten wir, dass Benadrin und Anergen dem Histamin in kleiner Menge in dieser Beziehung ähnelt, aber Minophagen C-Stark eine dem Histamin in grosser Menge entgegengesetzte Wirkung zeigt, während Neudrin eine gewisse Aehnlichkeit mit Adrenalin hat. Weiterhin bemerken wir hinsichtlich verschiedener anti-ulceröser Mittel, dass die Funktion des RES durch Larostidin, Umor, Stimulin M, Gastamin und Finalin gesteigert, aber durch Umor-Stark und Banthine unterdrückt wird.

In Bezug auf anti-canceröse Mittle berichteten Tomizuka usw.[10], dass Nitrogen-Mustard die Phagozytose und Wanderungsgeschwindigkeit der Leukozyten unterdrückt. Nach unseren Versuchsergebnissen wird aber die Funktion des RES durch Nitrogen-Mustard, Nitromin, Isoamyl- oder Cyclohexyl-Nitromin sowie Orthobromoparaaminotropolone gesteigert, doch seine Funktion bleibt unverändert durch Paraaminotropolone ohne anti-canceröse Wirkung.

An sonstigen Chemikalien beobachtete Fukunaga[11] eine gesteigerte Phagozytose der Leukozyten durch Lumin, und Bittersohl und Neidhardt[12] berichteten soger über eine Funktionssteigerung des RES durch Leberextrakt. Aus unseren Versuchsergebnissen wird weiter klar, dass die

Funktion des RES durch Lumin, Hikarutin, Spractor, Heparin, Greenpole, Solarson, Lebercalcin, Lebercalcin-Stark, Campolon und Curachihormon erhöht wird.

Schon in der V. Mitteilung[13] dieser Arbeit berichteten wir über eine Funktionssteigerung des RES durch Schilddrüsenhormon. Nach Astwood[14] wird angenommen, dass Methylthiouracil die Schilddrüsenfunktion unterdrückt. Aus obigen Versuchen bemerkten wir, dass die Funktion des RES durch Methiocil im Gegensatz zum Schilddrüsenhormon unterdrückt wird.

Infolgedessen wird durch obenerwähnte Versuchsergebnisse klargestellt, dass die grosse Mehrheit dieser Chemikalien reizend auf die Funktion des RES wirkt.

Zusammenfassung

Die Resultate obenerwähnter Unterschungen lassen sich folgendermassen zusammenfassen:

1. Von den verschiedenen Eiweisspräparaten rufen Eryosan, Jecuron, Mari-Amin, Vita-X, Neo-Minophagen AT, Neo-Soteira und Histamin in kleiner Menge, nicht aber Paniltin und Histamin in grosser Menge, eine Verminderung des Kongorotindex hervor, dagegen steigt die Prothrombinzahl nur durch Mari-Amin, Vita-X, Neo-Minophagen AT und Neo-Soteira an.

2. Von den verschiedenen anti-asthmatischen Mitteln führt Minophagen C-Stark eine Verminderung des Kongorotindex herbei, Benadrin sowie Anergen eine Verminderung mit nachheriger Vermehrung, während der Kongorotindex sich durch Neudrin vermehrt. Aber die Prothrombinzahl wird dabei in jedem Falle herabgesetzt.

3. Von den verschiedenen anti-ulcerösen Mitteln setzen Larostidin, Umor, Stimulin M, Gastamin und Finalin den Kongorotindex herab, aber Umor-Stark und Banthine erhöhen ihn. Die Prothrombinzahl wird aber nur durch Umor-Stark gesteigert.

4. Von den verschiedenen anti-cancerösen Mitteln verursachen Nitrogen-Mustard, Nitromin, Isoamyl-oder Cyclohexyl-Nitromin und Orthobromoparaaminotropolone, nicht aber Paraaminotropolone ohne anti-canceröse Wirkung, eine Verminderung des Kongorotindex, aber die Prothrombinzahl nimmt nur durch Isoamyl- oder Cyclohexyl-Nitromin zu.

5. Von den sonstigen Chemikalien rufen Lumin, Hikarutin, Spractor, Heparin, Greenpole, Solarson, Lebercalcin, Lebercalcin-Stark, Campolon und Curachihormon eine Verminderung des Kongorotindex, dagegen Methiocil dessen Vermehrung hervor, wobei die Prothrombinzahl nur durch Greenpole, Solarson und Campolon ansteigt.

6. Daraus ersieht man, dass die grosse Mehrheit dieser Chemikalien reizend auf die Funktion des RES Wirkt.

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Experimentelle Studien über die Funktion des reticuloendothelialen Systems

X. Mitteilung

Einfluss der experimentellen Pankreasschädigungen auf die Funktion des RES und der Leber

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Dass der Kohlehydratbestand der Leber abhängig ist von der Pankreastätigkeit, wird allgemein anerkannt, aber Arbeiten über Beziehung der Pankreasschädigungen zur Leberfunktion gibt es sehr wenig. Betreffs der Leberfunktion bei Diabetes mellitus bemerkte Kaito[1] ihre Herabsetzung mit nachfolgender Besserung durch Diättherapie und Tsuda[2] betonte sogar das gleiche Resultat bei alloxandiabetischen Kaninchen. Auch bei experimentellen Pankreatitiden berichteten einige Autoren über eine Herabsetzung der Leberfunktion (Ikushima[3], Yuasa[4], Yoshida[5], Kodama[6] und Nakamura[7]).

In Bezug auf die Funktion des RES bei Diabetes mellitus berichteten Ueda[8] und Unoura[9] über ihre Stabillität, und weiterhin beobachteten Leites und Riabow[10], dass die Funktion des RES bei Pankreasexstirpation unverändert bleibt.

Infolgedessen haben wir in der vorliegenden Untersuchung versucht wie Alloxandiabetes und experimentelle Pankreatitiden auf die Funktion des RES und der Leber einwirken.

Versuchsteil

Methodik

Die Bestimmungsmethode des Kongorotindex und der Prothrombinzahl war die gleiche wie bei der I. Mitteilung[11]. Um Alloxandiabetes zu verursachen, injizierten wir Alloxan (" Daiichi ") 200 mg pro kg in dis Ohrvenen der Kaninchen, die 24 Stunden lang gehungert hatten. Weiterhin haben wir experimentelle Pankreatitiden der Kaninchen hergestellt:

1) durch Unterbindung des Pankreasganges, 2) durch Injektion von Olivenöl in Menge von 0.3 ccm pro kg im Pankreasgang, und 3) durch Injektion von 10% Pankreatinlösung in Menge von 1 ccm pro kg im Pankreasgang.

Versuchsergebnisse

1. Einfluss der Alloxaninjektion

In der vorliegenden Untersuchung teilten wir alloxandiabetische Kaninchen in folgende 3 Formen ein: 1) eine vollkommene Form, wobei die Hyperglykämie über 200 mg/dl 4 Wochen lang andauert, 2) eine akute Form, wobei das Versuchstier in hochgradig hyperglykämischem Zustand binnen 8 Tagen stirbt, 3) eine unvollkommene Form, wobei der Blutzucker binnen 8 Tagen unter 200 mg/dl senkt.

Wie aus Tabelle I zu ersehen, vermindert sich der Kongorotindex und vermehrt sich die Prothrombinzahl in leichtem Grade bei Kontrollversuchen. Aber der Kongorotindex vermehrt sich bei vollkommener Form von Alloxandiabetes, während die Prothrombinzahl dabei fast unverändert bleibt. Weiterhin vermehrt sich der Kongorotindex bei akuter Form von Alloxandiabetes viel deutlicher als bei vollkommener Form, während die Prothrombinzahl sich in mässgem Grade vermindert. Im Gegensatz dazu vermehrt sich der Kongorotindex sowie die Prothrombinzahl bei unvollkommener Form von Alloxandiabetes.

Daraus wird klar, dass die Funktion des RES durch Alloxaninjektion bis zu einem gewissen Grade herabgesetzt wird, während die Leberzellenfunktion nur bei akuter Form von Alloxandiabetes zur Verminderung neigt.

2. Einfluss der antidiabetischen Mittel bei Alloxandiabetes

Schon in der V. und IX. Mitteilung[12] dieser Untersuchung berichteten wir, dass die Injektion von Redoxon (Vitamin C) in Menge von 50 mg pro kg eine Verminderung des Kongorotindex sowie eine Vermehrung der Prothrombinzahl hervorruft, dagegen die Injektion von Lebercalcin (Gluthationspräparat) in Menge von 2 mg pro kg nur eine Verminderung des Kongorotindex, während der Kongorotindex und die Prothrombinzahl durch Injektion von Insulin in Menge von 0.25 Einheiten (EH) pro kg fast unverändert bleiben.

Wie man aus Tabelle II ersieht, ruft die Injektion von Insulin, Redoxon oder Lebercalcin bei alloxandiabetischen Kaninchen eine leichte Verminderung des Kongorotindex mit nachfolgender Vermehrung hervor, während die Prothrombinzahl sich nur durch Insulin in leichtem Grade vermehrt.

Daraus geht hervor, dass die Funktion des RES durch antidiabetische Mittel wie Insulin, Vitamin C und Gluthation bei Alloxandiabetes in

TABELLE I

Einfluss der Alloxaninjektion

		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion									
				2 St.	4 St.	1 Tag	2 Tage	3 Tage	4 Tage	7 Tage	14 Tage	21 Tage	28 Tage
Kontroll	K-index	1	68.4	67.7	74.8	51.1	58.9	60.0	53.8	55.3	62.3	53.1	51.7
		2	62.8	61.8	72.1	55.4	50.0	56.2	60.4	60.4	45.7	55.2	55.0
		3	55.2	47.5	57.8	50.5	49.3	52.8	41.1	41.5	48.7	40.8	41.1
		4	47.7	48.3	60.0	51.3	44.4	56.2	50.8	44.6	49.5	45.8	50.0
		Durchschn.	58.8	56.6	66.8	52.2	51.1	56.4	51.7	51.3	51.7	48.7	48.7
		Differenz		-2.2	8.0	-6.6	-7.7	-2.2	-7.1	-7.5	-7.1	-10.1	-10.1
	P-zahl	1	1.00	1.00	1.14	1.10	1.16	1.19	1.19	1.15	1.00	0.98	1.15
		2	1.00	1.02	1.03	1.10	1.06	1.09	1.14	1.18	0.98	0.98	0.96
		3	1.00	1.00	1.03	1.02	1.06	1.09	1.17	1.02	1.04	1.00	0.98
		4	1.00	1.05	0.97	1.01	0.94	0.95	1.02	0.88	1.03	1.00	0.96
		Durchschn.	1.00	1.02	1.04	1.06	1.06	1.08	1.13	1.06	1.04	0.99	0.99
		Differenz		0.02	0.04	0.06	0.06	0.08	0.13	0.06	0.04	-0.01	-0.01
Vollkommene Form von Alloxan-diabetes	K-index	1	50.6	55.3	78.9	59.1	60.3	77.0	66.7	74.8	47.8	31.2	35.5
		2	44.8	88.8	66.6	41.3	45.3	84.6	75.2	50.0	58.8	50.6	42.9
		3	43.5	54.1	64.2	61.9	56.2	70.1	58.7	61.8	51.6	40.0	47.4
		Durchschn.	45.8	70.3	70.2	56.7	54.4	77.5	68.9	55.0	53.3	41.3	42.2
		Differenz		26.7	16.4	17.5	16.3	33.9	30.6	16.3	17.6	5.6	6.5
	P-zahl	1	1.00	1.08	0.98	1.16	1.08	1.21	1.23	1.08	1.11	1.10	1.04
		2	1.00	0.99	1.10	1.02	1.14	1.14	1.06	0.98	1.04	0.99	1.00
		3	1.00	0.93	0.96	0.98	1.05	1.00	1.01	1.03	1.00	1.00	0.94
		Durchschn.	1.00	1.00	1.01	1.05	1.09	1.16	1.10	1.03	1.03	1.03	0.99
		Differenz		-0.02	-0.03	-0.01	0.03	0.08	-0.03	-0.01	-0.01	0.04	0
Akute Form von Alloxan-diabetes	K-index	1	66.1	80.2	88.8	71.2	59.0	82.0	+				
		2	35.4	68.2	68.7	65.2	76.0	82.4	75.0	78.6	+		
		3	33.0	67.5	46.5	52.1	65.7	62.0	+				
		Durchschn.	45.1	72.2	69.7	62.3	69.3	77.5					
		Differenz		29.3	17.6	23.8	31.9	34.6					
	P-zahl	1	1.00	0.93	0.93	0.91	0.96	1.16	+				
		2	1.00	0.98	0.99	1.03	1.02	1.06	1.05	1.05	+		
		3	1.00	0.92	0.96	0.95	0.92	1.01	+				
		Durchschn.	1.00	0.94	0.96	0.96	0.97	1.08					
		Differenz		-0.08	-0.08	-0.10	-0.09	0					
Unvollkommene Form von Alloxan-diabetes	K-index	1	46.1	59.9	60.0	55.1	77.0	63.6	61.4	55.3	36.4		
		2	40.5	50.0	54.8	46.3	42.7	50.8	41.5	43.0	32.9		
		3	34.8	40.0	48.1	53.9	33.7	39.3	39.6	34.8	32.2		
		Durchschn.	40.6	50.3	54.8	52.2	48.3	51.5	47.6	44.3	34.1		
		Differenz		11.9	6.2	18.2	15.4	13.1	14.1	11.2	0.6		
	P-zahl	1	1.00	1.05	1.01	0.96	1.25	1.30	1.22	1.15	1.18		
		2	1.00	1.00	0.98	1.11	1.24	1.17	1.16	1.11	1.13		
		3	1.00	1.00	0.93	0.92	1.11	1.10	1.19	0.94	1.15		
		Durchschn.	1.00	1.02	0.97	1.00	1.20	1.19	1.19	1.08	1.15		
		Differenz		0	-0.05	-0.06	0.14	0.11	0.06	0.02	0.11		

TABELLE II

Einfluss der antidiabetischen Mittel bei alloxandiabetes

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Insulin	0.25 EH s.c.	K-index	1	36.2	41.6	61.0	60.2
			2	31.9	29.2	30.2	38.7
			Durchschn.	34.1	35.4	46.0	49.2
			Differenz		-2.2	7.6	11.4
		P-zahl	1	1.00	1.06	1.02	1.07
			2	1.00	1.03	1.02	1.03
			Durchschn.	1.00	1.05	1.03	1.05
			Differenz		0.04	0	0.02
Redoxon	50 mg i.v.	K-index	1	44.4	49.2	58.7	56.7
			2	35.5	33.7	37.9	42.3
			Durchschn.	39.5	40.7	47.0	48.7
			Differenz		-2.1	3.2	5.5
		P-zahl	1	1.00	0.96	0.98	0.99
			2	1.00	0.94	0.94	0.92
			Durchschn.	1.00	0.95	0.96	0.96
			Differenz		-0.06	-0.07	-0.07
Lebercalcin	2 mg s.c.	K-index	1	57.7	44.8	54.4	54.2
			2	34.7	36.3	41.9	38.6
			Durchschn.	40.3	40.4	47.5	44.9
			Differenz		-3.2	2.9	0.9
		P-zahl	1	1.00	0.94	0.95	0.94
			2	1.00	0.91	0.87	0.89
			Durchschn.	1.00	0.93	0.91	0.92
			Differenz		-0.08	-0.12	-0.12

leichtem Grade gereizt wird, während nur die Insulininjektion eine Funktionssteigerung der Leberzellen verursacht.

3. Einfluss der experimentellen Pankreatitiden

Wenn man die Bauchhöhle des Versuchskaninchens ohne Narkose öffnet, so geschieht, wie Tabelle III zeigt, eine leichte Vermehrung des Kongorotindex, während die Prothrombinzahl fast unverändert bleibt. Aber die Unterbindung des Pankreasganges ruft dabei eine leichte Vermehrung des Kongorotindex sowie eine leichte Verminderung der Prothrombinzahl hervor und die Injektion von Olivenöl im Pankreasgang dabi eine Vermehrung des Kongorotindex sowie eine Verminderung der Prothrombinzahl in mässigem Grade, während die Pankreatininjektion im Pankreasgang eine deutliche Vermehrung des Kongorotindex sowie eine starke Verminderung der Prothrombinzahl verursacht.

Aus diesen Versuchen wird klar, dass die Funktion des RES und der Leberzellen durch experimentelle Pankreatitiden bis zu einem gewissen Grade herabgesetzt wird.

TABELLE III

Einfluss der experimentellen pankreatitiden

		Nr. des Kaninchens	Vor Op.	Zeit nach Operation (St.)							
				3	5	8	12	24	48	72	96
Laparotomie	K-index	1	67.8	69.6	67.5	80.1	76.8	70.4	41.6	77.6	48.9
		2	60.4	60.6	70.0	69.0	79.0	66.6	63.1	73.3	66.6
		3	59.1	61.7	63.0	56.3	70.6	60.3	34.5	82.7	78.9
		4	43.9	66.6	59.1	50.0	62.6	79.1	73.9	38.8	49.2
		5	38.5	46.9	68.4	66.7	67.6	41.1	54.1	34.1	45.3
		Durchschn.	53.2	59.8	66.3	65.0	71.6	62.5	53.0	58.2	55.2
		Differenz		6.6	13.1	11.8	18.4	9.3	-0.2	5.0	2.0
	P-zahl	1	1.00	1.03	1.09	1.15	1.13	1.09	1.22	1.08	1.02
		2	1.00	0.99	0.90	0.98	1.04	1.04	1.20	0.99	1.01
		3	1.00	0.96	1.04	1.02	0.93	0.91	0.92	1.15	1.14
		4	1.00	0.96	0.95	1.01	0.97	0.96	1.09	1.00	1.07
		5	1.00	0.93	1.01	1.04	1.09	0.99	1.00	1.01	0.94
		Durchschn.	1.00	0.97	0.98	1.04	1.03	1.00	1.09	1.05	1.04
		Differenz		-0.03	-0.02	0.04	0.03	0	0.09	0.05	0.04
Unterbindung des Pankreasganges	K-index	1	60.0	71.0	60.8	71.0	76.8	67.4	59.4	46.7	62.5
		2	38.5	69.1	65.0	74.2	78.0	78.6	72.2	50.0	63.5
		Durchschn.	49.3	70.2	63.2	70.8	77.3	73.0	65.1	48.2	63.0
		Differenz		14.3	0.8	9.7	9.6	14.4	16.0	-6.1	11.5
	P-zahl	1	1.00	0.99	0.88	0.95	0.97	1.15	1.08	1.08	0.90
		2	1.00	0.97	0.92	0.97	0.97	0.97	1.06	0.98	1.00
		Durchschn.	1.00	0.98	0.90	0.96	0.97	1.06	1.07	1.03	0.95
		Differenz		0.01	-0.08	-0.08	-0.06	-0.02	-0.02	-0.02	-0.09
Olivenöl-injektion im Pankreasgang	K-index	1	54.1	82.5	80.0	78.0	63.1	60.4	48.1	68.4	51.7
		2	50.0	75.0	90.4	63.5	76.0	81.1	68.5	51.1	37.0
		Durchschn.	56.5	80.3	84.8	70.2	69.3	70.6	58.0	60.4	44.1
		Differenz		17.2	15.2	1.9	-5.6	4.8	1.7	-1.1	-14.4
	P-zahl	1	1.00	0.97	0.94	0.94	1.00	1.03	1.16	0.87	0.95
		2	1.00	0.91	0.95	0.92	0.99	0.99	1.05	0.94	0.90
		Durchschn.	1.00	0.94	0.95	0.93	0.97	1.01	1.11	0.91	0.93
		Differenz		-0.03	-0.03	-0.11	-0.06	0.01	0.02	-0.14	-0.11
Pankreatin-injektion im Pankreasgang	K-index	1	41.6	57.1	80.7	81.5	78.3	76.9	43.5	37.1	32.3
		2	33.9	71.4	65.5	76.0	80.0	85.3	50.6	+	
		Durchschn.	38.9	63.0	72.8	78.8	79.2	78.7	46.9		
		Differenz		17.5	20.8	28.1	21.9	30.5	8.2		
	P-zahl	1	1.00	0.91	1.00	0.91	1.05	0.93	0.97	1.11	1.14
		2	1.00	0.92	0.88	0.89	0.99	0.95	1.13	+	
		Durchschn.	1.00	0.92	0.94	0.90	1.02	0.94	1.05		
		Differenz		-0.05	-0.04	-0.14	-0.01	-0.06	-0.04		

DISKUSSION

Es ist schon bekannt, dass die Leberfunktion sowohl bei Diabetes mellitus als auch bei Alloxandiabetes bis zu einem gewissen Grade herabgesetzt wird (Kaito[1], Tsuda[2]). Betreffs der Funktion des RES bei Diabetes mellitus gibt es aber nur wenige Arbeiten. Ueda[8] und Unoura[9]

berichteten, dass die Funktion des RES bei Diabetespatienten fast unverändert bleibt und weiterhin beobachteten Leites und Riabow[10], dass die Funktion des RES bei Pankreasexstirpation auch unverändert bleibt.

Aus unseren Versuchsergebnisse geht hervor, dass die Funktion des RES durch Alloxaninjektion bis zu einem gewissen Grade herabgesetzt wird, während die Leberzellenfunktion nur bei akuter Form von Alloxandiabetes zur Verminderung neigt.

Aber die bisherigen Ergebnisse über Insulinwirkung auf die Funktion des RES sind sehr verschieden: viele Autoren berichteten über eine Funktionssteigerung des RES durch Insulin (Goldzieher[13], Leites[10], Haendel[14], Samaras[15]), umgekehrt Saxl und Donath[16] über eine Herabsetzung, während Kinoshita[17] und Uchino[18] betonten, dass die Funktion des RES durch Insulin in grosser Menge abnimmt, aber durch nachfolgende Insulininjektion in kleiner Menge wieder zunimmt. In Bezug auf antidiabetische Mittel, abgesehen von Insulin, beobachteten Stepp u.a.[19] eine Unterdrückungseffekt des Blutzuckers von Vitamin C, und weiterhin bemerkte Lazarow[20] eine Schutzwirkung gegen Alloxandiabetes von Gluthation. Schon in der V. und IX. Mitteilung[12] dieser Unteruchung berichteten wir, dass Insulin in Menge von 0.5 oder 2 EH pro kg, Redoxan in Menge von 50 mg pro kg und Lebercalcin in Menge von 2 mg pro kg die Funktion des RES der gesunden Kaninchen steigert, nicht aber Insulin in Menge von 0.25 EH pro kg, dass aber die Leberzellenfunktion nur durch Redoxon erhöht wird.

Aus unseren Versuchsergebnisse ist festzustellen, dass die Funktion des RES durch antidiabetische Mittel wie Insulin, Redoxon und Lebercalcin bei Alloxandiabetes in leichtem Grade gereizt wird, während die Funktion der Leberzellen sich nur durch Insulin vermehrt.

In Bezug auf die Leberfunktion bei experimentellen Pankreatitiden berichteten Ikushima[3], Yuasa[4], Yoshida[5], Kodama[6] und Nakamura[7] über ihre Herabsetzung, aber es gibt dafür keine Untersuchung über die Funktion des RES.

Nach unseren diesbezüglichen Ergebnissen ist festzustellen, dass die Funktion des RES und der Leberzellen bei experimentellen Pankreatitiden durch Unterbindung des Pankreasganges sowie Injektion von Olivenöl oder Pankreatin im Pankreasgang, bis zu einem gewissen Grade herabgesetzt wird.

Aus obenerwähnten Versuchsergebnissen ist zu vermuten, dass das Pankreas, nicht nur als inkretorisches Organ, sondern auch als exkretorisches Organ, an der Funktion des RES und der Leberzellen beteiligt ist.

Zusammenfassung

Fassen wir obige Versuchsergebnisse zusammen, so kommen wir zu folgendem Schluss:

1. Der Kongorotindex vermehrt sich bei vollkommener Form von Alloxandiabetes, während die Prothrombinzahl fast unverändert bleibt. Weiterhin vermehrt sich der Kongorotindex bei akuter Form davon vie deutlicher als bei vollkommener Form, während die Prothrombinzahl sich in mässigem Grade vermindert. Im Gegensatz dazu vermehrt sich der Kongorotindex sowie die Prothrombinzahl bei unvollkommener Form von Alloxandiabetes.

2. Antidiabetische Mittel wie Insulin, Redoxon und Lebercalcin rufen eine leichte Verminderung des Kongorotindex mit nachfolgender Vermehrung hervor, während die Prothrombinzahl sich nur durch Insulin in leichtem Grade vermehrt.

3. Experimentelle Pankreatitiden durch Unterbindung des Pankreasganges und Injektion von Olivenöl oder Pankreatin im Pankreasgang verursachen eine Vermehrung des Kongorotindex sowie eine Verminderung der Prothrombinzahl, wobei die Veränderung durch Unterbindung des Pankreasganges am leichtesten und die durch Pahkreatininjektion im Pankreasgang am stärksten ist.

4. Daraus ist zu vermuten, dass das Pankreas, nicht nur als inkretorisches Organ, sondern auch als exkretorisches Organ, an der Funktion des RES und der Leberzellen beteiligt ist.

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A Not on the Colorimetric Ratio of Uric Acid to Adrenaline in the Folin, Cannon and Denis' Phosphotungstic Acid Method

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In the estimation of adrenaline by the phosphotungstic acid method of Folin, Cannon and Denis,[1] uric acid is used as the reference standard. And usually, the colorimetric ratio of uric acid to adrenaline is taken as 1:3 according to the estimate of Folin *et al.*[1] It is questionable, however, whether the adrenaline preparation used by Folin *et al.* in order to compare with uric acid was the chemically purest, as it was recently demonstrated by Goldenberg *et al.*,[2] Auerbach and Angell[3] and Tullar[4] that some of the commercial adrenaline preparations contained a considerable amount of noradrenaline.

In the present investigation the colorimetric ratio of uric acid to adrenaline in the Folin, Cannon and Denis' method was re-examined, using synthetic L-adrenaline (Sankyo Co.) and uric acid (Merck). The ratios obtained in 10 experiments were 1:2.97–1:3.03 and the mean value was 1:3.00. This value is nearly equal to that by Folin *et al.* (1:2.90–1:2.95) and that by Johannessohn[5] (1:2.98), but a little higher than that by Autenrieth and Quantmeyer[6] (1:3.3).

In addition, the colorimetric ratio of uric acid to a mixture of adrenaline and noradrenaline was determined. It was estimated to be 1:2.85 in a mixture of 10% noradrenaline and 90% adrenaline; 1:2.67 in a mixture of 20% noradrenaline and 80% adrenaline; 1:2.39 in a mixture of 30% noradrenaline and 70% adrenaline, and so on. Therefore, it is reasonable to assume that the low adrenaline value of the commercial adrenaline preparations indicated in the papers of Stewart and Rogoff[7] and of Kodama[8] may be due to the presence of noradrenaline in the adrenaline preparations.

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Experimentelle Studien über die Funktion des reticuloendothelialen Systems

XI. Mitteilung

Einfluss von Erwärmung und Badekur auf die Funktion des RES und der Leber

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Es unterliegt keinem Zweifel, dass das Wärmezentrum den von Temperaturänderungen bedingten Reiz auf dem Wege des vegetativen Nervenzentrums beantwortet. Vor allem betonte Hoff[1], dass das vegetative Nervensystem bei Badekur zweiphasich reagiert, und zwar erstens sympathicotonisch, zweitens parasympathicotonisch. Aus der III. Mitteilung[2] dieser Arbeit ist es klargelegt geworden, dass das vegetative Nervensystem in inniger Beziehung steht zur Funktion des RES und der Leberzellen. Aber die Angaben der Forscher sind sehr unzulänglich betreffs der Veränderungen des RES und der Leber im Erregungszustand des Wärmezentrums.

Infolgedessen haben wir es uns zur Aufgabe gemacht, zu untersuchen, welchen Einfluss Erwärmung und Badekur auf die Funktion des RES und der Leber ausüben.

Versuchsteil

Methodik

Die Auswahl der Versuchstiere sowie die Bestimmungsmethode der Funktion des RES und der Leberzellen erfolgten genau so wie bei der früheren Mitteilung[3]. Die Erwärmung des Versuchskaninchens wurde im gut ventilierten Wärmeschrank oder im heissen Wasserbad vorgenommen, die Temperaturen schwankten zwischen 40°–42°C, und die Feuchtigkeit zwischen 45–50%. Die Dauer der Ueberheitzung im Wärmeschrank oder im heissen Wasserbad betrug von 15 Minuten bis 5 Stunden, und die Tiere wurden im Versuchskasten 5 Stunden lang gehalten.

Versuchsergebnisse

1. Einfluss der langdauernden Erwärmung

Wenn man Versuchskaninchen 5 Stunden lang im Wärmeschrank erwärmt, so geschieht, wie Tabelle I zeigt, eine Verminderung des Kongorotindex mit nachfolgender Vermehrung, während die Prothrombinzahl fast unverändert bleibt. Es kommt zu demselben Resultat auch bei wechselnder Erwärmung in Zwischenräumen von 15 Minuten. Aber die intravenöse Injektion von 25% Glukose in Menge von 10 ccm pro kg verursacht dabei eine mässige Verminderung des Kongorotindex, während

TABELLE I

Einfluss der langdauernden Erwärmung

	Dose (pro kg)		Nr. des Kaninchens	Vor Erwärmung	Zeit nach Erwärmung (St.)		
					1	3	5
Erwärmung		K-index	1	54.3	58.2	68.2	66.7
			2	45.8	53.8	78.5	69.3
			Durchschn.	50.2	56.1	73.2	68.0
			Differenz		-5.9	3.9	0.3
		P-zahl	1	1.00	1.06	1.01	0.99
			2	1.00	0.96	0.96	0.99
			Durchschn.	1.00	1.01	0.99	0.99
			Differenz		0.01	0.01	-0.01
Glukose bei Erwärmung	25% 10 ccm i.v.	K-index	1	46.9	56.3	60.0	62.5
			2	43.3	58.0	45.4	47.5
			Durchschn.	45.1	57.1	52.3	55.3
			Differenz		0.3	-12.3	-7.3
		P-zahl	1	1.00	1.00	0.99	0.97
			2	1.00	1.00	0.97	0.97
			Durchschn.	1.00	1.00	0.98	0.97
			Differenz		0.01	0.01	-0.01
Lävulose bei Erwärmung	25% 10 ccm i.v.	K-index	1	41.7	37.5	38.2	41.8
			2	36.7	47.8	54.7	60.0
			Durchschn.	38.7	42.7	47.0	50.8
			Differenz		-7.8	-10.8	-5.4
		P-zahl	1	1.00	1.09	1.05	1.06
			2	1.00	1.07	1.05	1.05
			Durchschn.	1.00	1.08	1.05	1.06
			Differenz		0.09	0.08	0.08
Wechselnde Erwärmung in Zwischenräumen von 15 Minuten		K-index	1	59.6	68.7	76.6	82.4
			2	47.3	52.7	61.4	59.3
			Durchschn.	54.2	62.0	70.3	74.0
			Differenz		-6.0	3.2	9.6
		P-zahl	1	1.00	1.00	1.00	1.02
			2	1.00	0.99	0.99	0.96
			Durchschn.	1.00	1.00	1.00	0.99
			Differenz		0	0.01	-0.03

die Prothrombinzahl unverändert bleibt. Dagegen veranlasst die Injektion von 25% Lävulose in Menge von 10 ccm pro kg eine starke Verminderung des Kongorotindex und eine starke Vermehrung der Prothrombinzahl.

Daraus ist erkennbar, dass die Funktion des RES sowohl bei langdauernder als auch bei wechselnder Erwärmung sich von der 1. bis zur 3. Stunde erhöht, im späteren Stadium aber gestört wird und dass die obige Funktionsstörung des RES und der Leberzellen späterhin durch Lävulose beseitigt wird, während Glukose nur die Funktion des RES verbessert.

2. Einfluss der kurzfristigen Erwärmung

Wie aus Tabelle II erkennbar ist, verursacht eine Erwärmung von 15 Minuten im Wärmekasten oder im heissen Wasserbad eine Verminderung des Kongorotindex mit nachfolgender Vermehrung, während die Prothrombinzahl sich vermindert. Aber ein Thermalbad mit Tamagawa-Mineralwasser (der salzsäurehaltigen schwefelsauren Eisenoxydulquelle in der Akita-Präfektur), ruft eine Verminderung des Kongorotindex und eine Vermehrung der Prothrombinzahl hervor, dagegen veranlasst ein Schwefelblütenbad der Tamagawa-Quelle eine Verminderung des Kongorotindex und der Prothrombinzahl.

Aus diesen Versuchen geht hervor, dass kurzfristige Erwärmung im Wärmeschrank und im heissen Wasserbad eine erhebliche Steigerung der Funktion des RES herbeiführt, dagegen wird die Leberzellenfunktion nur durch Tamagawa-Mineralwasserbad gebessert.

3. Einfluss verschiedener Arten von Mineralwasser

Wie aus Tabelle III zu ersehen ist, ruft ein Thermalbad mit Genzoyu-Mineralwasser (der schwefelwasserstoffhaltigen sauren Quelle des Badeortes Narugo in der Miyagi-Präfektur) oder mit Unagiyu-Mineralwasser (der kochsalzhaltigen alkalischen Quelle des Badeortes Narugo) eine Verminderung des Kongorotindex und eine Vermehrung der Prothrombinzahl hervor, in ähnlicher Weise wie ein Thermalbad mit Tamagawa-Mineralwasser.

Schon in der III. Mitteilung[2)] dieser Forschung berichteten wir, dass die Atropininjektion eine Zunahme des Kongorotindex, im Gegensatz dazu die Ergotamininjektion seine Abnahme hervorruft. Aber man sieht aus Tabelle IV, dass die obenerwähnte Verminderung des Kongorotindex sowie die Vermehrung der Prothrombinzahl nicht durch Atropin im Menge von 1 mg pro kg beeinflusst werden, während die beiden durch Ergotamin in Menge von 1 mg pro kg ganz gehemmt werden.

Aus diesen Versuchsergebnissen lässt sich erkennen, dass Thermalbäder verschiedener Quellen die Funktion des RES sowie der Leberzellen steigert

TABELLE II

Einfluss der kurzfristigen Erwärmung

		Nr. des Kaninchens	Vor Erwärmung	Zeit nach Erwärmung (St.) 1	3	5
Erwärmung von 15 Minuten	K-index	1	37.8	47.2	52.7	58.2
		2	36.4	35.1	41.6	50.0
		Durchschn.	37.1	40.8	47.1	54.0
		Differenz		-10.1	-2.7	6.7
	P-zahl	1	1.00	1.00	0.99	0.97
		2	1.00	0.99	0.99	0.90
		Durchschn.	1.00	1.00	0.99	0.94
		Differenz		0	0	-0.08
Bad von 15 Minuten	K-index	1	42.8	44.5	54.3	68.0
		2	37.4	38.7	48.9	51.6
		Durchschn.	40.1	42.6	51.5	60.3
		Differenz		-12.3	-1.2	10.2
	P-zahl	1	1.00	0.98	0.96	1.00
		2	1.00	0.97	1.00	0.92
		Durchschn.	1.00	0.98	0.98	0.96
		Differenz		-0.02	0.01	-0.06
Bad mit Tamagawa-Mineralwasser	K-index	1	52.8	44.7	54.0	65.2
		2	40.5	49.2	47.8	53.2
		Durchschn.	47.0	46.8	51.3	58.5
		Differenz		-14.0	-8.4	1.3
	P-zahl	1	1.00	1.02	1.09	1.04
		2	1.00	1.05	1.05	1.04
		Durchschn.	1.00	1.04	1.07	1.04
		Differenz		0.04	0.08	0.02
Schwefelblütenbad der Tamagawa-Quelle	K-index	1	57.2	60.3	58.6	63.8
		2	41.7	49.2	52.7	57.0
		Durchschn.	50.2	55.9	56.2	61.1
		Differenz		-8.1	-6.7	0.7
	P-zahl	1	1.00	1.03	0.92	0.88
		2	1.00	0.96	0.88	0.93
		Durchschn.	1.00	1.00	0.90	0.91
		Differenz		0	-0.09	-0.11

und dass die obige Wirkung bei Tamagawa-Mineralwasserbad durch den Sympathicus stimuliert wird.

DISKUSSION

Dass die Leberfunktionsstörung wegen Wärmestauung im Lebensverhältnis der hohen Temperaturen entsteht, ist von manchen Autoren anerkannt (Sakurai[4], Takano[5], Kawakatsu[6], Yoshinaka[7], Saito[8], Oda[9], Kato und Tokuhiro[10]). Nach Saito[8] ist es aber klar geworden, dass die Leberfunktionsstörung infolge der Erwärmung leichtgradiger ist als die

TABELLE III

Einfluss verschiedener Mineralwässer

		Nr. des Kaninchens	Vor Baden	Zeit nach Baden (St.)		
				1	3	5
Bad mit Tamagawa-Mineralwasser	K-index	1	52.8	44.7	54.0	65.2
		2	40.5	49.2	47.8	53.2
		Durchschn.	47.0	46.8	51.3	58.5
		Differenz		-14.0	-8.4	1.3
	P-zahl	1	1.00	1.02	1.09	1.04
		2	1.00	1.05	1.05	1.04
		Durchschn.	1.00	1.04	1.07	1.04
		Differenz		0.04	0.08	0.02
Bad mit Genzoyu-Mineralwasser	K-index	1	48.7	38.1	59.5	61.5
		2	42.0	41.7	47.3	51.6
		Durchschn.	45.2	39.7	53.8	56.7
		Differenz		-18.3	-4.7	0.7
	P-zahl	1	1.00	1.02	1.04	1.07
		2	1.00	1.04	1.05	1.02
		Durchschn.	1.00	1.03	1.04	1.04
		Differenz		0.04	0.07	0.06
Bad mid Unagiyu-Mineralwasser	K-index	1	63.4	69.5	76.3	46.7
		2	57.0	63.0	68.0	74.2
		Durchschn.	60.2	66.6	73.0	67.4
		Differenz		-7.4	0.1	-3.0
	P-zahl	1	1.00	1.07	1.09	1.04
		2	1.00	1.07	0.98	1.03
		Durchschn.	1.00	1.07	1.03	1.04
		Differenz		0.08	0.06	0.06

durch Arzneimittelinjektion oder Wärmestich. Weiterhin beobachteten manche Forscher (Sakurai[4], Takano[5], Kawakatsu[6], Saito[8]), dass die Leberfunktion im frühen Stadium der Erwärmung gefördert und im späten gestört wird. In Bezug auf die Funktion des RES durch Erwärmung sind indessen die Angabe der Autoren sehr ungenügend. Muda[11] bemerkte eine Funktionssteigerung des RES durch Erwärmung, und Lüdke[12] sowie Tawara[13] bestätigten weiter eine Steigerung der Produktion der Agglutinine durch Erhitzung.

Unsere Versuchsergebnisse stellen klar, dass die Funktion des RES sowohl bei langdauernder als auch bei kurzfristiger Erwärmung von der 1. bis zur 3. Stunde gesteigert, aber im späteren Stadium gestört und diese Funktionsstörung dann durch Glukose und Lävulose wieder gebessert wird.

Hinsichtlich des Einflusses der Badekur auf die Funktion des RES und der Leber berichtete Todoroki[14], dass die Funktion des RES durch einmaliges Baden herabgesetzt, dann aber durch fortlaufende Badekur erhöht

TABELLE IV

Einfluss der vegetativen Nervengifte auf die Wirkung von Tamagawa-Mineralwasser

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Bad mit Tamagawa-Mineralwasser		K-index	1	52.8	44.7	54.0	65.2
			2	40.5	49.2	47.8	53.2
			Durchschn.	47.0	46.8	51.3	58.5
			Differenz		-14.0	-8.4	1.3
		P-zahl	1	1.00	1.02	1.09	1.04
			2	1.00	1.05	1.05	1.04
			Durchschn.	1.00	1.04	1.07	1.04
			Differenz		0.04	0.08	0.02
Bad mit Tamagawa-Mineralwasser nach Atropin	1 mg s.c.	K-index	1	48.9	50.0	55.8	57.1
			2	46.4	45.3	46.4	57.2
			Durchschn.	47.5	47.6	51.7	57.3
			Differenz		-13.7	-8.5	-0.4
		P-zahl	1	1.00	1.03	1.04	1.04
			2	1.00	0.88	1.00	1.04
			Durchschn.	1.00	0.96	1.02	1.04
			Differenz		-0.04	0.03	0.06
Bad mit Tamagawa-Mineralwasser nach Ergotamin	1 mg s.c.	K-index	1	50.8	50.0	64.4	83.3
			2	44.5	48.5	60.0	56.7
			Durchschn.	47.7	49.2	62.7	73.5
			Differenz		-12.3	2.3	15.6
		P-zahl	1	1.00	0.96	0.81	0.92
			2	1.00	0.93	0.93	0.90
			Durchschn.	1.00	0.95	0.87	0.91
			Differenz		-0.05	-0.12	-0.11

wird. Ferner bemerkte Ikeda[15] und Yamagami[16], dass die phagozytäre Kraft der Histiozyten unter der Haut durch Badekur mehr gesteigert wird als durch gewöhnliches warmes Bad.

Aus unseren Versuchsergebnissen geht hervor, dass Thermalbäder mit Tamagawa-, Genzoyu- und Unagiyu-Mineralwässern die Funktion des RES sowie der Leberzellen in weit hoherem Masse steigern als ein gewöhnliches warmes Bad.

Betreffs des Einflusses der Badekur auf das vegetative Nervensystem berichtete Hoff[1], dass dieses auf Badekur zweiphasisch reagiert und der heilsame Einfluss der Badekur auf die erstphasische Sympathicotonie zu beschränken ist. Aber Ito[17] und Matsuo[18] behaupteten, dass die Heilkraft der Badekur in der zweitphasischen Parasympathicotonie zu suchen ist.

Unsere Versuchsergebnisse bestätigen also, dass die Funktionssteigerung des RES sowie der Leberzellen beim Baden mit Tamagawa-Mineral-

wasser durch den Sympathicus gesteigert wird.

Zusammenfassung

Auf Grund unserer Versuchsresultate können wir wie folgend schliessen:

1. Eine 5 Stunden lange oder eine wechselnde Erwärmung in Zwischenräumen von 15 Minuten ruft eine Verminderung des Kongorotindex mit nachfolgender Vermehrung hervor, während die Prothrombinzahl fast unverändert bleibt. Dabei verursacht die Lävuloseinjektion eine starke Verminderung des Kongorotindex und eine starke Vermehrung der Prothrombinzahl, während die Glukoseinjektion nur eine Verminderung des Kongorotindex veranlasst.

2. Eine 15 Minuten lange Erwärmung im Wärmeschrank oder im heissen Wasserbad lässt den Kongorotindex geringer werden und später wieder zunehmen, während die Prothrombinzahl abnimmt.

3. Die Thermalbäder mit Tamagawa-, Genzoyu- und Unagiyu-Mineralwässern vermindern den Kogorotindex und vermehren die Prothrombinzahl in höherem Grade als ein gewöhnliches heisses Bad, während ein Schwefelblütenbad der Tamagawa-Quelle eine Verminderung des Kongorotindex und der Prothrombinzahl verursacht.

4. Weil der obenerwähnte Effekt von Tamagawa-Mineralwasser nicht durch Atropin, sondern durch Ergotamin beseitigt wird, so folgt daraus eindeutig, dass die obige heilsame Wirkung durch den Sympathicus stimuliert wird.

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Spectroscopic Study on the Interaction of Amino Acids and Sugars*

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The reddish purple colour which occurs when acetylhexosamine once heated in dilute alkali is treated with the Ehrlich's aldehyde reagent, has been utilized for the determination of hexosamine[1]. As is known, however, such glucidamins as seromucoid, loach mucin[2] and gastric mucin immediately give the positive colour reaction with the Ehrlich reagent. The colour produced here is similar to the one above but only a single absorption maximum is shown at 550 mμ[3]. Furthermore, Vasseur and Immers[4] recently discovered that product of sugars after interaction with amino compounds react with Ehrlich's aldehyde reagent, developing colour resembling the one produced from alkali-treated acetylhexosamine. The present investigation has been undertaken with the aim to compare the absorptoin spectra of those colours.

Experimental

Method

Reagents

1) *p*-Dimethylaminobenzaldehyde (*p*-DAB) reagent.— A. R. grade *p*-dimethylaminobenzaldehyde was purified by distillation in vacuo and then fractionated by addition of water to 50–70% to its ethanol solution. The crystals appearing thereby were almost colourless. The reagent was prepared by dissolving 1 g. of the material in a mixture of 90 m*l*. of glacial acetic acid and 10 m*l*. of concentrated hydrochloric acid (sp. gr. 1.18). It was coloured pale yellow.

2) 1/3 *N* Na_2CO_3.

3) Alkaline acetylacetone solution.—0.1 m*l*. of fractionally distilled acetylacetone (b.p. 136–137°C.) was added to 15 m*l*. of 1/3 *N* Na_2CO_3 solution.

* Read before the Annual Meeting of the Japanese Biochemical Society, Tokyo, April 26–28, 1953.

Spectrophotometry

It was affected in automatic-recording spectrophotometer (Hitachi) over the wave-length range 400–760 mμ at room temperature (13–16°C.). Cell 1 cm. thick.

The Colour Reaction with *p*-DAB of Substances obtained by Interaction of Amino Acids and Sugars

Glycine, L-alanine, DL-serine, L-leucine, L-tyrosine, DL-tryptophane, L-glutamic acid sodium salt, L-lysine hydrochloride, L-arginine hydrochloride, and taurine were let react with sugars such as glucose, galactose, arabinose and glucosamine hydrochloride.

An appropriate amount of those amino acids were weighed in 15× 115 mm. test tubes.

Then 1 m*l*. of 20% solution of the sugars were separately poured into the test tubes to treat by "Osaki-Turumi's indirect process"[3]. Namely, after addition of 2 m*l*. of the alkaline solution, the solutions were heated in a boiling water bath for 5 minutes, when they turned yellowish brown. They were then cooled under running water and 4 m*l*. of *p*-DAB reagent were added, and the mixtures here were heated in boiling water for 30 seconds, whereby a purplish red colour developed, and cooled in water to room temperature. Followingly each was transferred to a 10 m*l*. volumetric flask and made up to the mark with the *p*-DAB reagent. Different sugars did not show any remarkable divergency among the wave-lengths of absorption maximum.

When 10 mg. or so of glycine, alanine, serine, methionine or tyrosine were used as an amino acid, the absorption curves showed the absorption maximum all at about 520 mμ and were of similar feature except for serine. Fig. 1 illustrates the case of leucine (10 mg. or so). The curve of serine in Fig. 2 has a step at 570–580 mμ. On the other hand, lysine, arginine, and taurine in an amount less than the amino acids gave a deep reddish purple colour. Lysine (0.2 mg.)- and taurine (2.5 mg.)-compounds showed similar, comparatively sharp absorption curves, the maximum for lysine-compound lying at 576 mμ, and that for taurine-compounds lying at about 580 mμ.

In Fig. 3 is shown the curve for lysine. Arginine (9.8 mg.)-compounds gave absorption curves with maximum at about 574 mμ and a step at 540–560 mμ. The results are shown in Fig. 4. Tryptophane and glucosamine treated in the same way gave rise to red coloration without any absorption maximum. See Fig. 5.

The Colour Reaction of Tryptophane and Indol

As is known, tryptophane gives the positive Ehrlich reaction due to

Fig. 1

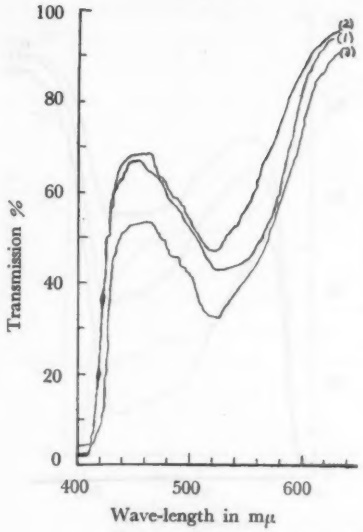

Fig. 2

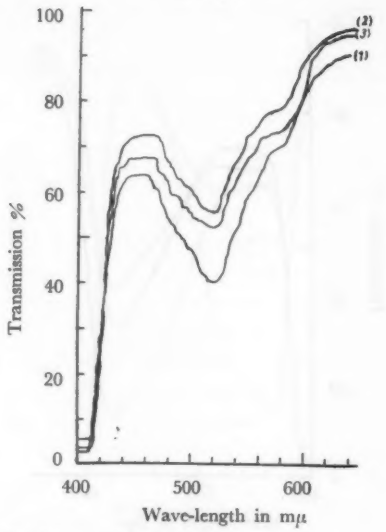

Fig. 1. The absorption spectra of mixtures of leucine and various sugars after treatment with Na_2CO_3 and *p*-DAB reagent. (1) Mixture of leucine (10.4 mg.) and 1 m*l*. of glucose (20%); (2) mixture of leucine (9.6 mg.; and 1 m*l*. of galactose (20%); (3) mixture of leucine (9.8 mg.) and 1 m*l*. of galactose (20%).

Fig. 2. The absorption spectra of mixtures of serine and various sugars after treatment with Na_2CO_3 and *p*-DAB reagent. (1) Mixture of serine (9.8 mg.) and 1 m*l*. of glucose (20%); (2) mixture of serine (9.8 mg.) and 1 m*l*. of galactose (20%); (3) mixture of serine (10.0 mg.) and 1 m*l*. of arabinose (20%).

indol nucleus. 5 mg. of tryptophane were dissolved in 3 m*l*. of water and treated with *p*-DAB reagent as above, without previous heating with alkali. Two absorption maxima were shown at about 550 mμ and 574 mμ, and a minimum at about 564 mμ. The curve obtained after treatment of 0.05 mg. of indol had a single maximum at about 572 mμ. The product from indol absorbed light more intensively than that from tryptophane. The details are seen in Fig. 6.

The Colour Reaction of N-Acetylglucosamine and Glucosamine

The reddish purple colour, which develops when acethylhexosamine is treated with Na_2CO_3 and Ehrlich's reagent, has been utilized by Elson-Morgan for colorimetric determination of acetylhexosamines[1]. Examination of the absorption curves in that reaction was undertaken under the

Fig. 3

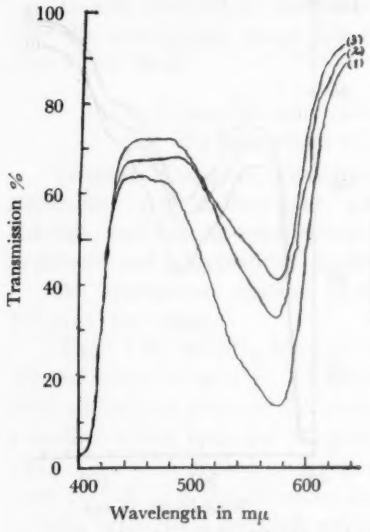

Fig. 3

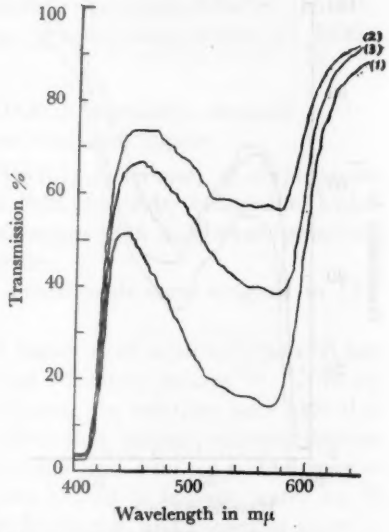

Fig. 3. The absorption spectra of mixtures of lysine hydrochloride and various sugars after treatment with Na_2CO_3 and *p*-DAB reagent. 0.2 mg. portions of lysine hydrochloride were mixed separately with 1 m*l*. of glucose (20%) (1), 1 m*l*. of galactose (20%) (2), and 1 m*l*. of arabinose (20%) (3).

Fig. 4. The absorption spectra of mixtures of arginine hydrochloride and various sugars after treatment with Na_2CO_3 and *p*-DAB reagent. (1) Mixture of arginine-HCl (9.6 mg.) and 1 m*l*. of glucose (20%); (2) mixture of arginine-HCl (9.8 mg.) and 1 m*l*. of galactose (20%); (3) mixture of arginine-HCl (9.8 mg.) and 1 m*l*. of arabinose (20%).

same conditions, as follows.

0.5 mg. of N-acetylglucosamine was dissolved in 1 m*l*. of water, and after addition of 2 m*l*. of the alkali, heated in boiling water for 5 minutes, followed by treatment with *p*-DAB. The absorption spectrum possessed two maxima at about 548 mμ and 586 mμ, and a minimum at about 570 mμ. But, when 2–3 mg. of glucosamine hydrochloride were treated in the same way, excepting that the alkaline acetylacetone solution was replaced for the Na_2CO_3 solution, the absorption curve showed only a single maximum at about 546 mμ. These curves are seen in Fig. 7.

Comment

1) It has been found that monoamino acids give with sugars products, whose absorption curves all possess an absorption maximum around 520 mμ. But, such amino acids as taurine, lysine etc. gave dyes with an absorption

Fig. 5

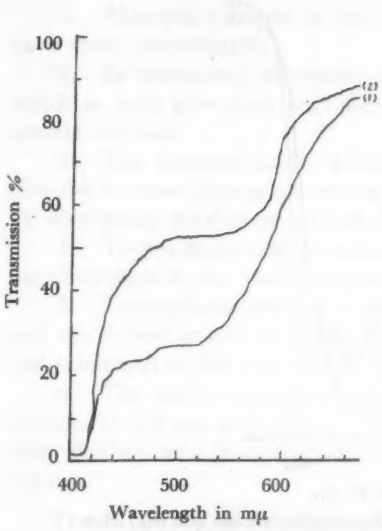

Fig. 6

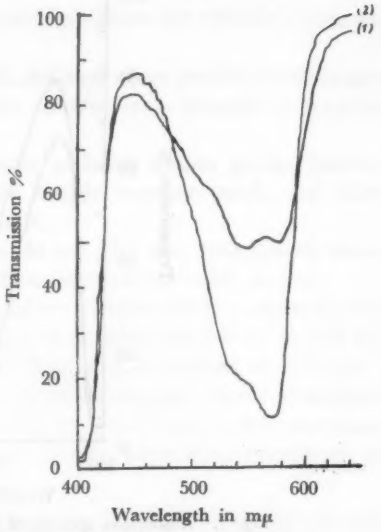

Fig. 5. The absorption spectra of mixtures of glucose and tryptophane, and of glucose and glucosamine hydrochloride, after treatment with Na_2CO_3 and *p*-DAB reagent. (1) Mixture of tryptophane (10 mg.) and 1 m*l*. of glucose (20%); (2) mixture of glucosamine-HCl (10.6 mg.) and 1 m*l*. of glucose (20%).

Fig. 6. Absorption spectra of (1) indole (0.05 mg.) and (2) tryptophane (5.0 mg.) after treatment with *p*-DAB reagent.

maximum at about 576 mμ or so. According to Kubota, α-amino acids react with glucose at the same rate, but the reaction velocity of β-amino acids is greater than that of α-amino acid because of inhibitory action of the carboxyl group[5]. Further, Vasseur and Immers noticed that β-amino acids gave far more intense colour reaction than α-amino acids[4]. It is plausively assumed that α-amino groups react with sugars in one and the same manner, but amino groups at other positions not only have a stronger reactivity but also produce a substance different from the one by the α-amino groups.

Although Vasseur and Immers described that alanine, serine, methionine, tyrosine, and glutamic acid give no colour reaction[4], our experiments showed that these amino acids give a faint red colour. Moreover, according to Immers and Vasseur, the absorption maxima when lysine and glucose interact lie at 560–570 mμ,[6] and according to Aminoff *et al.*[7] at about 560 mμ, but the present experiment showed it at about 576 mμ.

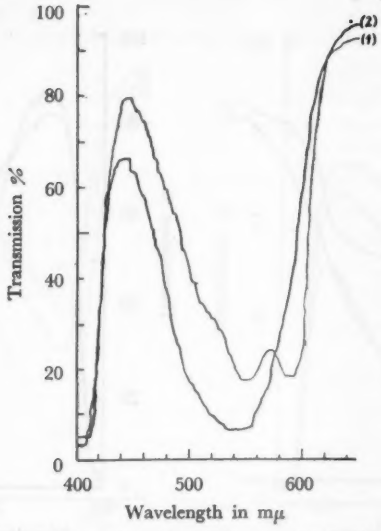

Fig. 7. Absorption spectra of (1) N-acetylglucosamine (0.5 mg.) after treatment with Na_2CO_3 and *p*-DAB reagent, and (2) glucosamine hydrochloride (2.3 mg.) after treatment with alkaline acetylacetone and *p*-DAB reagent.

2) The absorption curves for arginine and serine had a step. This seems to be caused by the guanido-group of arginine and the hydroxyl-group of serine in the amino acids. Tryptophane does not show any characteristic absorption, when it reacts with sugars, while it gives the colour reaction with Ehrlich's reagent immediately without any prior alkali-treatment, giving a coloured solution with absorption maximum at 574 mμ, that is, near to the maximum of that after the corresponding reaction of indol.

Concerning N-acetylglucosamine, Osaki and Turumi reported that it gives two absorption maxima at 540 mμ and 586 mμ[8], Aminoff *et al.* at 550 mμ and 590 mμ[7] and Gottschalk and Lind at 544 mμ and 587 mμ[8]. The data obtained by us are almost identical with the findings of the two last groups of investigators. On treatment with alkaline acetylacetone solution glucosamine gives a coloured product with a single absorption maximum at 544 mμ, which suggests difference between the coloured substance produced by N-acetylglucosamine and that by glucosamine treated with alkaline acetylacetone solution.

A hypothesis regarding the chemical structure of chromogens in these reactions will be expressed elsewhere.

SUMMARY

1. Absorption curves of the coloured products by Ehrlich's reaction have been investigated.

2. By interacting of α-amino acids and sugars are produced substances which in turn give dyes with Ehrlich's reagent with absorption maxima around 520 mμ.

3. The α-amino acids, which have another amino group besides, give rise to more intense coloration than simple α-amino acids, and show an absorption maximum around 576 mμ.

4. Tryptophane and glucosamine do not give any compounds showing absorption in the visible region, when they interact with sugars.

5. Tryptophane and indole give positive Ehrlich reaction immediately, and the former shows an absorption spectrum with maximum at 550 mμ and minimum at 564 mμ, but the latter that with maximum at 572 mμ.

6. The colour developed by N-acetylglucosamine shows absorption maxima at 548 mμ and 586 mμ, while that by glucosamine after treatment with alakline acetylacetone possesses one single absorption maximum at 544 mμ.

Thanks are due to Mr. Masao Sumino of the Central Laboratory, Hitachi Co. for his enthusiastic co-operation in the course of this work.

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Studies on Brain Stem

IX. Comparative Anatomical Study of Brain Stems of some Species of Bats

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(Received for publication, March 10, 1954)

In our previous "Studies on Brain Stem VIII. On the Positions of Abducens Nucleus and Abducens Root of Birds"[1], we have pointed out that in birds the abducens nucleus and its root characteristically appear rather to the caudal side than the facial nucleus and its root. Now, the bats of course belong to the mammalian kingdom, but they are one with birds in their habit of flying with their patagia. With reference to this habit of flying, we tried to investigate the influence of this habit on the anatomy of the brain stem including the relative positions of the abducens and the facial nuclei, in comparison with birds on the one hand and with other mammalia incapable of flying on the other.

As materials, we used Pal-carmine stained serial transverse sections of the brains of a South American vampire bat (desmodus rufus), a Japanese greater horseshoe bat (rhinolophus), an Indian tuber-nosed bat (harpyiocephalus leucogostra) and an Indian kalong (pteropus edulis), which we examined serially from the caudal side upwards to the cranial.

Experimental

Findings

Vampire bat (*desmodus rufus*): The abducens nucleus and its root make their appearance at a level far more caudal than the appearance of the facial nucleus and its root, indeed at the same level with the fila radicularia n. vagi et glossopharyngici. The pyramidal tract is almost unobservable both while the abducens nucleus is visible and in the sections more cranial to it. The olivary nucleus is seen on either side of the rhaphe in good development, occupying the position of the pyramidal tract in other mammalia. The vestibular nucleus is not so strongly developed as in birds, but

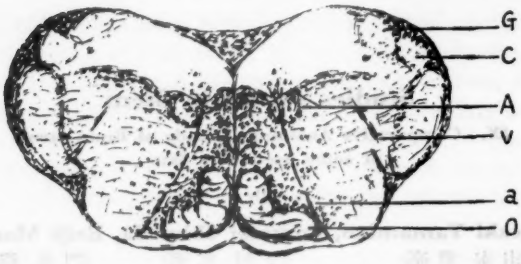

Fig. 1. Transversal section of brain stem in vampire bat, Pal-carmine staining, low power view. A abducens nucleus; a abducens root; v fila radic. n vagi et glossoph.; O olivary nucleus; G gracile nucleus; C cuneate nucleus.

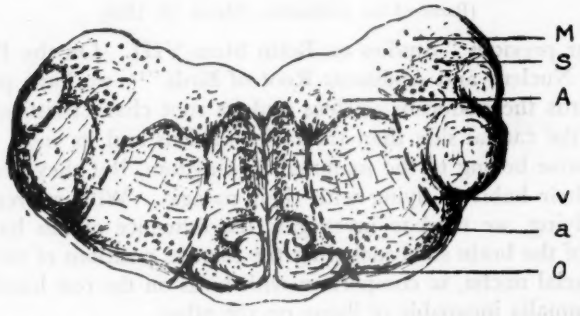

Fig. 2. Transversal section of brain stem in greater horseshoe hat, Pal-carmine staining, low power view. A abducens nucleus; a abducens root; v fila radic. n. vagi et glossoph.; O olivary nucleus; M medial vestibular nucleus; S spinal vestibular nucleus.

better than in other non-flying mammalia. The development of this nucleus was appreciated by ocular estimation of the ratio of the area it takes in against the total cross-area of the section.

Greater horseshoe bat (*rhinolophus*): Similar to the case of the vampire bat above, the abducens nucleus and its root appear much more caudally than the facial nucleus and its root, at the same level with the fila radicularia n. vagi et glossopharyngici. The facial nucleus becomes visible only after the abducens nucleus has entirely disappeared from the field. The pyramidal tract is very weak in development, so that we were almost led to deny its existence, its proper position being taken up by the olivary nucleus. The vestibular nucleus was found comparatively well developed.

Tuber-nosed bat (*harpyiocephalus leucogostra*): The abducens nucleus be-

gins to appear at the ventro-lateral side of the genu of the facial root as the facial nucleus almost goes out of sight, similar to other mammalia in this respect. At the level where the facial nucleus disappears completely, the abducens nucleus does not attain full development. The pyramidal tract is considerably well developed. On the level where the olivary nucleus comes into view on the caudal side, the pyramidal tract exists on the ventral side of the olivary nucleus close to it, adjacent to either side of the rhaphe; the olivary nucleus is not situated on the lateral side of the pyramidal tract, as in other mammalia, but adjacent to the latter in vertical positions on either side of the rhaphe. As we go up more cranially, the olivary nucleus gradually develops, so that the pyramidal tract is pushed by to the lateral side, only a very small portion remaining on the ventral

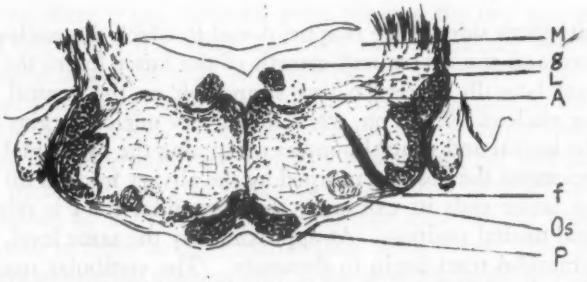

Fig. 3. Transversal section of brain stem in tuber-nosed bat, Palcarmine staining, low power view. A abducens nucleus; Os superior olive; g genu of facial root; f facial root; L lateral vestibular nucleus; M medial vestibular nucleus; P pyramidal tract.

side of the olivary nucleus in a posture as if crushed down by the latter. At more cranial levels, when the olivary nucleus begins to decrease in size, the pyramidal tract on the lateral side of it begins to invade the space occupied by the olivary nucleus, while the portion crouched down on the ventral side thereof begins to enlarge. These two strands of the pyramidal tract show the appearance of two independent tracts, the one coming back from the lateral position taking in the more dorsal position. Soon after the facial nucleus makes its appearance, the dorsal bundle first decussates, the ventral bundle decussating at the rostral level. The vestibular nucleus is not better developed than in other mammalia in any marked degree.

Indian kalong (*pteropus edulis*): The abducens nucleus and its root begins to appear more cranially than the facial nucleus and its root. The pyramidal tract is rather strongly developed and its relation to the olivary nucleus is similar to that of the organs in the tuber-nosed bat described above: before the appearance of the olivary nucleus, the pyramidal tract is

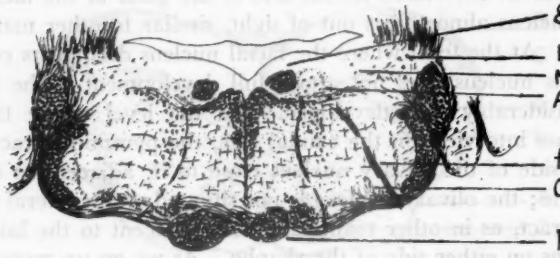

Fig. 4. Transversal section of brain stem in kalong, Pal-carmine staining, low power view. A abducens nucleus; Os superior olive; g genu of facial root; f facial root; L lateral vestibular nucleus; M medial vestibular nucleus; P pyramidal tract.

found lining both sides of the rhaphe, dorsal to which the nucleus makes its appearance and the subsequent growth of the latter forces the larger part of the tract laterally, except a tiny strand left on the ventral side of the expanding nucleus. Later on, when the olivary nucleus begins to diminish in size, the lateral and ventral bundles composing the pyramidal tract come back to reassume the position usurped by the olivary nucleus till at the level where the latter ends its existence, the pyramidal tract is reinstituted in the original medial position. At approximately the same level, the strands of the pyramidal tract begin to decussate. The vestibular nucleus in this kind of bats is of ordinary development, not stronger than in other non-flying mammalia in any sense.

Discussion

The four species of chiroptera studied above are not much different in their ecology, including their common habit of aviation, though some difference may exist in their food habit. However, it is of interest to note that the four fall into two istinct groups in view of the structure of their brain stem; the one comprises the vampire and the greater horseshoe bats, the other the tuber-nosed bat and the kalong. In the first group, the abducens nucleus appears at a more caudal level than the facial nucleus—an arrangement similar to the characteristical relative positions in birds. It is accepted that the birds have no pyramidal tracts; in this group of bats, the pyramidal tract does exist, but in an extremely weak development, almost only in a trace of it. The vestibular nucleus, the nucleus of equilibrium, is also much better developed in this group than in other non-flying species of mammalia, though, maybe, in a lesser degree than in birds. Such observations indicate a brain stem anatomy much closer to birds than to ordinary mammalia.

On the other hand, in the specimens belonging to the latter group, the relative positions of the abducens nucleus and the facial nucleus are quite the ame as in other families of mammalia, the abducens nucleus coming into view at a level near the cranial end of the facial nucleus. The pyramidal tract is rather well developed, while the vestibular nucleus is not so unusually developed as in the first group.

Bats, both of the first and the second groups above, are all one in their birdlike habit and capacity of flying through the air, and it has been established by Spillmann[2], Koike[3] and others that their patagia come into form in early embryonic stage, and yet, interestingly enough, only four specimens studied by us showed that they are divisible into two distinct groups, one with the anatomy of the brain stem nearer to birds than other mammalian species and the other quite vice versa.

However, there is one common point between the two groups, that is, the mutual relation of the pyramidal tract and the olivary nucleus. The olivary nucleus is located close to either side of the rhaphe and is rather well developed in both groups, but in the first group comprising the vampire bat and the greater horseshoe bat, the pyramidal tract is almost rudimentary, which in the second group comprising the tuber-nosed bat and the kalong, where the pyramidal tract is rather well developed, it is side-tracked to a lateral position by the olivary nucleus, so that their relative positions are just the reverse of those in other mammalian brain stems. The pyramidal tract recovers its original position only at the level where the olivary nucleus has ceased to exist. It may be said that the pyramidal tract has surrendered its right of way to the olivary nucleus as far as the latter continues to exist. This fact seems to indicate that, in bats, the olivary nucleus takes precedence over the pyramidal tract in the anatomy of the brain stem, showing here also an affinity to birds in this respect. As Brehm[4] has pointed out, the bats are not at all inferior to birds in the ability and delicate aptitude of their flight, and it is no wonder that the olivary nucleus belonging to the extrapyramidal tract takes in a superior position over the pyramidal tract in bats.

Anyhow, it may be clearly noted that the observations on the brain stems of bats show some points of resemblance in their anatomy to those of birds.

Summary

1. The abducens nucleus, the pyramidal tract and the vestibular nucleus of a vampire bat and a greater horseshoe bat show an arrangement more resembling birds rather than other members of mammalia.

2. These organs of a tuber-nosed bat and kalong are almost identical

with other non-chiropteral mammalia in their positions and development.

3. The olivary nucleus takes precedence over the pyramidal tract in all the four specimens of bats studied.

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Noradrenaline and Adrenaline Contents of Suprarenal Glands in Various Species of Animals

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I have reported in detail a method to estimate noradrenaline and adrenaline separately by using the permanganate reagent. Further, I have assayed noradrenaline and adrenaline contents of the medulli-suprarenal extracts of cattle and horses with this method[1].

The present investigation was undertaken to extend this study to the suprarenal extracts of other mammals, such as pigs, goats, dogs, cats and rabbits.

Experimental

Methods

The suprarenal glands of pigs, goats, dogs, cats and rabbits were used. The glands of pigs and goats were taken out as soon as possible after the slaughter of the animals at the abattoir. In cases of dogs, cats and rabbits, the animals were killed by blows on the neck and the glands were removed immediately after death. These glands were extracted with 4% trichloracetic acid.

The differential assay of noradrenaline and adrenaline content of the acid extracts was performed by using our permanganate method.[2] This method depends on the fact that noradrenaline is oxidized with the permanganate reagent in different oxidation rates corresponding to the different pH. The details have been described in another paper[1] of mine.

As the reference standard, synthetic L-adrenaline base of Sankyo Co. was used. This was dissolved with N/10 HCl in 1:1,000 and kept in a refrigerator.

Results

The experimental results are presented in Tables I-V.

1) *Pig Suprarenals*

With the extracts of 11 glands, weighing 1.82–2.70 g., noradrenaline

and adrenaline contents were estimated.

The data obtained in these estimations are tabulated (Table I).

TABLE I

Pig Suprarenals

No. of exp.	No. of animal and sex	Side of gland	Weight of gland (g.)	Noradrenaline mg. per g. of gland	Adrenaline mg. per g. of gland	Per cent noradrenaline
1	1 ♂	r	1.82	1.06	1.25	46
2		l	2.20	1.07	1.05	50
3	2 ♂	r	2.33	0.52	1.20	30
4		l	2.37	0.48	1.10	30
5	3 ♀	r	2.25	0.83	0.77	52
6		l	2.30	0.70	0.89	44
7	4 ♂	r	2.70	0.55	0.84	39
8	5 ♀	r	2.20	0.84	0.92	48
9		l	2.10	1.02	0.99	51
10	6 ♀	r	2.20	0.71	0.87	45
11		l	2.50	0.47	0.77	38
Max.				1.07	1.25	52
Min.				0.47	0.77	30
Mean				**0.75**	**0.97**	**43**

The noradrenaline contents per g. of gland were 0.47–1.07 mg. and the average was 0.75 mg. The amounts of adrenaline per g. of gland varied between 0.77 and 1.25 mg., the average being 0.97 mg. Accordingly, the relative amounts of noradrenaline were 30–52 per cent and the mean value was 43 per cent.

2) *Goat Suprarenals*

With the acid extract of 15 glands, weighing 0.43–1.30 g., the amounts of noradrenaline and adrenaline were assayed. The data are given in Table II.

The noradrenaline contents were estimated to be 0.32–1.75 mg. per g. of gland, the average being 0.83 mg. The adrenaline contents per g. of gland were 1.44 (0.84–2.95) mg. The relative amount of noradrenaline was calculated to be 36.5 (21–68) per cent.

3) *Dog Suprarenals*

8 dogs, weighing 6.7–12.1 kg., were experimented on. The whole glands of suprarenals from each dog were extirpated immediately after death. And then, each gland was weighed and extracted with trichloracetic acid. In one dog, however, only right gland was extracted, because the sister gland was partially injured during its extirpation. With the extract

TABLE II

Goat Suprarenals

No. of exp.	No. of animal and sex	Side of gland	Weight of gland (g.)	Noradrenaline mg. per g. of gland	Adrenaline mg. per g. of gland	Per cent noradrenaline
1	1 ♀	r	1.200	0.77	1.80	30
2		l	1.300	0.61	1.67	26
3	2 ♀	r	0.600	0.66	1.37	32
4		l	0.620	0.72	1.39	34
5	3 ♀	r	0.670	0.55	1.27	30
6		l	0.800	0.32	1.19	21
7	4 ♀	r	0.950	1.75	0.84	68
8	5 ♀	r	0.560	0.82	1.24	40
9		l	0.596	0.68	1.10	38
10	6 ♂	r	0.430	1.14	1.26	47
11		l	0.473	0.97	1.23	44
12	7 ♀	r	0.669	1.05	2.95	26
13		l	0.744	0.97	2.16	31
14	8 ♂	r	0.551	0.63	1.03	38
15		l	0.474	0.81	1.06	43
Max.				1.75	2.95	68
Min.				0.32	0.84	21
Mean				**0.83**	**1.44**	**36.5**

of 15 glands, weighing 0.408–0.853 g. the colorimetric determinations were performed. The data are shown in Table III.

The noradrenaline estimates were 0.11–0.27 mg. per g. of gland and the mean value was 0.18 mg. The adrenaline contents were estimated to be 0.87 (0.47–1.60) mg. Accordingly, the relative amount of noradrenaline was 18.5 (7–29) per cent.

4) *Cat Suprarenals*

7 cats, weighing 2.08–4.18 kg., were used. The whole glands from each animal were extirpated immediately after death. The glands of both sides were weighed and extracted together. With 7 extracts of both glands, weighing 0.278–0.496 mg., colorimetric determinations were performed. The data obtained in 7 estimations are displayed in Table IV.

The noradrenaline contents per g. of gland were estimated to be 0.24–0.85 mg., the average being 0.50 mg. The adrenaline estimates were 0.28–1.10 mg. per g. of gland and the mean value was 0.61 mg. The values per kg. of body weight were 0.058 (0.032–0.079) mg. for noradrenaline and 0.069 (0.045–0.100) mg. for adrenaline. Accordingly, the relative amount of noradrenaline was 45.1 (38–53) per cent.

TABLE III

Dog Suprarenals

No. of exp.	No. of animal Body weight & sex	Side of gland	Weight of gland (g.)	Noradrenaline mg. per g. of gland	Adrenaline mg. per g. of gland	Per cent noradrenaline
1	1					
	9.5 kg. ♀	r	0.707	0.14	0.47	23
2	2	r	0.724	0.15	0.66	18
3	6.7 kg. ♀	l	0.601	0.11	0.95	10
4	3	r	0.629	0.15	0.63	19
5	8.6 kg. ♀	l	0.613	0.17	0.73	19
6	4	r	0.754	0.17	0.70	20
7	8.7 kg. ♀	l	0.707	0.26	0.81	24
8	5	r	0.853	0.25	0.62	29
9	10.0 kg. ♀	l	0.678	0.27	0.86	24
10	6	r	0.585	0.17	0.76	19
11	11.7 kg. ♂	l	0.581	0.18	0.81	18
12	7	r	0.411	0.20	0.90	18
13	7.1 kg. ♂	l	0.408	0.24	1.10	18
14	8	r	0.628	0.18	1.51	11
15	12.1 kg. ♂	l	0.615	0.11	1.60	7
Max.				0.27	1.60	29
Min.				0.11	0.47	7
Mean				**0.18**	**0.87**	**18.5**

TABLE IV

Cat Suprarenals

No. of exp.	Body weight & sex	Weight of glands (g.) l+r	Noradrenaline		Adrenaline		Per cent noradrenaline
			mg. per g. of gland	mg. per kg. of body weight	mg. per g. of gland	mg. per kg. of body weight	
1	2.42 kg ♀	0.407	0.41	0.069	0.39	0.066	51
2	2.08 kg ♀	0.331	0.29	0.046	0.28	0.045	51
3	2.80 kg ♀	0.430	0.48	0.074	0.65	0.100	42
4	3.79 kg ♂	0.430	0.49	0.055	0.69	0·078	41
5	3.28 kg ♀	0.303	0.85	0.079	0.77	0.071	53
6	4.18 kg ♂	0.278	0.73	0.049	1.10	0.073	40
7	3.70 kg ♂	0.496	0.24	0.032	0.39	0.052	38
Max.			0.85	0.079	1.10	0.100	53
Min.			0.24	0.032	0.28	0.045	38
Mean			**0.50**	**0.058**	**0.61**	**0.069**	**45.1**

5) *Rabbit Suprarenals*

6 rabbits, weighing 2.16–3.36 kg., were used. The suprarenal glands of both sides in each rabbit were removed immediately after death, weighed and extracted together with trichloracetic acid. With 6 extracts of both glands, weighing 0.264–0.480 g., the colorimetric determinations were performed. The results are reproduced in tabular form (Table V).

TABLE V

Rabbit Suprarenals

No. of exp.	Body weight & sex	Weight of glands (g.) l+r	Noradrenaline		Adrenaline		Per cent noradrenaline
			mg. per g. of gland	mg. per kg. of body weight	mg. per g. of gland	mg. per kg. of body weight	
1	2.60 kg. ♀	0.264	0	0	0.48	0.049	0
2	2.16 kg. ♀	0.273	0	0	0.29	0.037	0
3	2.28 kg. ♀	0.416	0	0	0.20	0.036	0
4	2.62 kg. ♀	0.342	0	0	0.35	0.045	0
5	3.36 kg. ♀	0.427	0.05	0.006	0.39	0.050	11
6	2.36 kg. ♀	0.480	0	0	0.29	0.058	0
Max.			0.05	0.006	0.48	0.058	11
Min.			0	0	0.20	0.036	0
Mean			**0.008**	**0.001**	**0.33**	**0.046**	**1.8**

The adrenaline contents per g. of gland were 0.20–0.48 mg., the average being 0.33 mg. Computing per kg. of body weight, they were 0.046 (0.036–0.058) mg. On the other hand, noradrenaline was found in only one extract to be contained in an amount of 0.05 mg. per g. of gland and 0.006 mg. per kg. of body weight.

Accordingly, the relative amount of noradrenaline was calculated to be 1.8 (0–11) per cent.

DISCUSSION

In my experiments mentioned above, noradrenaline and adrenaline contents in the suprarenal extracts of various species of animals such as pigs, goats, dogs, cats and rabbits were assayed colorimetrically. All the data are epitomized in a summary table (Table VI). At a glance of this table, it will be noticed that the values varied considerably according to the animal species.

The averages of the relative amounts of noradrenaline in my experimental results were 43 per cent in pigs, 36.5 per cent in goats, 18.5 per cent in dogs, 45.1 per cent in cats and 1.8 per cent in rabbits. Namely, in my experiments it was observed that the suprarenal extracts of cats, pigs and

TABLE VI

Summary Table

Noradrenaline and Adrenaline Contents in Suprarenal Glands of Various Kinds of Animals

	Noradrenaline mg. per g. of gland	Adrenaline mg. per g. of gland	Per cent noradrenaline
Pig	0.75 (0.47 - 1.07)	0.97 (0.77 - 1.25)	43 (30 - 52)
Goat	0.83 (0.32 - 1.75)	1.44 (0.84 - 2.95)	36.5 (21 - 68)
Dog	0.18 (0.11 - 0.27)	0.87 (0.47 - 1.60)	18.5 (7 - 29)
Cat	0.50 (0.24 - 0.85)	0.61 (0.28 - 1.10)	45.1 (38 - 53)
Rabbit	0.008 (0 - 0.05)	0.33 (0.20 - 0.48)	1.8 (0 - 11)

goats contain noradrenaline in a considerably large percentage in addition to adrenaline, those of dogs in somewhat smaller proportion and those of rabbits in a negligibly small per cent.

As regards the noradrenaline content of the suprarenal glands in various kinds of mammals, numerous investigations have been reported.[3)-12)] Here, the estimates of my experiments will be compared with those of previous investigators.

In the suprarenal extract of pigs, noradrenaline was found by Holtz & Schümann[3)] to be contained in about 30 per cent of the total active substances. Their value was somewhat lower than that of my experiments. In the paper of Shepherd & West[4)], it was reported that noradrenaline was contained in the average in an amount of 49 per cent of the total, which was almost the same with my estimate.

It was estimated by Bülbring & Burn[5)] that noradrenaline was absent from two out of five extracts of dog suprarenals and that its relative amount in three other extracts was 14, 28 and 52 per cent, respectively. These were also reported in their other paper[6)], adding that two other glands were found to contain only adrenaline. Bülbring[7)] found that the suprarenals of dogs contained 0–2.0 mg. noradrenaline and 0.5–1.3 mg. adrenaline per g. of gland. According to the investigation of Schuler & Heinrich[8)], noradrenaline and adrenaline per g. of dog suprarenal gland were 0.688 mg. and 0.978 mg., respectively, and the relative amount of noradrenaline was 41 per cent. Shepherd & West[9)] reported that noradrenaline in the adrenals of adult dogs was found to be 27 per cent of the total catechol amines. Therefore the relative amount of noradrenaline estimated in my experiment was somewhat smaller than that of Shepherd

& West and much less than that of Schuler & Heinrich.

In the suprarenal glands of cats noradrenaline and adrenaline were found by Euler[10], in amounts of 0.2–1.1 mg. and 0.2–1.4 mg. per g. of glands, respectively. He also reported that the relative amount of noradrenaline was 24–78 per cent. In the investigations of Bülbring[7], the suprarenals of cats were found to contain 0.4–3.7 mg. noradrenaline and 0.3–1.1 mg. adrenaline per g. of gland. Schuler & Heinrich[8] reported that noradrenaline and adrenaline content per g. of gland was 0–0.908 mg. and 0.170–1.197 mg., respectively, and the relative amount of noradrenaline was 42 (0–81) per cent. And also, according to Shepherd & West[9], the mean value of noradrenaline content was 0.369 mg. per g. of gland and that of adrenaline was 0.601 mg. The relative amount of noradrenaline was 38 per cent in the mean value. Thus, concerning the relative amount of noradrenaline, the data of these previous investigators agreed approximately with those obtained in my experiments.

It was found by Schuler & Heinrich[8] that the suprarenal extracts of rabbits contained 0.33–1.10 mg. noradrenaline per g. of gland and 0.756–1.65 mg. adrenaline, and the relative amount of noradrenaline was 34 (17–53) per cent. Hökfelt & McLean reported that the suprarenal gland of rabbit contained 0–0.07 mg. noradrenaline and 0.27–0.49 mg. adrenaline per g. and that the relative amount of noradrenaline was 0–13 per cent of the total. According to the investigations of Shepherd & West[9], the mean value of noradrenaline content was 0.01 mg. per g. of gland and that of adrenaline was 0.47 mg. Accordingly, only 2 per cent of the total catechol amines were noradrenaline. Holtz & Schümann[5] reported that no arterenol was detected in rabbit suprarenals. So far as the rabbit suprarenal glands were concerned, the estimates obtained in my experiments of noradrenaline content per g. of gland as well as the relative amount of noradrenaline were considerably lower than those of Schuler & Heinrich and were in accordance with those of Hökfelt & McLean, Shepherd & West and Holtz & Schümann.

Summary

By using the permanganate method, noradrenaline and adrenaline contents of suprarenal extracts in pigs, goats, dogs, cats and rabbits were assayed. It was estimated that the relative amount of noradrenaline in the suprarenals was 43 (30–52) per cent of the total active substances in pigs, 36.5 (21–68) per cent in goats, 18.5 (7–29) per cent in dogs, 45.1 (38–53) per cent in cats and 1.8 (0–11) per cent in rabbits, respectively.

My thanks are due to Prof. T. Suzuki for his advice given during the course of this work.

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Nucleic Acids

VII. A Combination of Several Methods for Determination of Purine and Pyrimidine Bases in DNA with Minor Modifications

By

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The paper-chromatographic analysis of purine and pyrimidine bases begun by Vischer & Chargaff[1] and Hotckiss[2] has been elaborated step by step and particularly Markham & Smith[3] and Wyatt[4] have contrived advantageous techniques for separating the bases from one another on a single paper sheet. The writers tried to combine best devices regarding individual stages in the predecessors' methods which all consisted of hydrolysis of a DNA, separation of the bases on paper, extraction of the bases and measurement of the extinctions, with simplifications.

1) To begin with, concentrated formic acid is most profitable, as will be accounted for in Discussion below, to liberate the bases quantitatively from nucleic acids excepting uracil which is not found in DNA.

2) Regarding irrigation, the writers examined various butanol-containing solvents by ascending chromatography on a Toyo Roshi filter paper No. 3. However, none of the solvents were found to be able to well separate all the bases by a single irrigation, as is exemplified in Table I, in accord with the experiences of Vischer & Chargaff[1] and of Hotckiss.[2] Because of this difficulty, the former authors proposed to develop chromatograms of purines and of pyrimidines separately and the latter author to distinguish the individual bases with their characteristic ultraviolet absorption spectra. Much superior to those solvents was found Wyatt's solvent isopropanol-conc. HCl (sp. gr. 1.18)-water (70:17:13 by volume), giving Rf values of adenine, guanine, cytosine, thymine and uracil of 0.43, 0.31, 0.54, 0.86 and 0.72 respectively on Toyo Roshi filter paper No. 3 at 26°C.

3) To detect spots on the chromatogram, Makino & Matsuzaki's method[6] is preferrable to Markham & Smith's[7] for the convenience that the spots can be seen with the naked eyes.

4) The solvent for the extraction must be as low acidic as possible, as will be explained below. And it is advisable to employ 0.1 *N* HCl to

TABLE I

Rf Values of Purine and Pyrimidine Bases Run with Various Butanol-containing Solvents

Paper slivers 2.8 cm.×45 cm. were used. They were spotted with watery solutions of the bases on a horizontal line ruled 7 cm. from the bottom. Solvent front run 35 cm. Temperature of test 22-26°C.

No.	Solvent Composition by volume		Adenine	Guanine	Cytosine	Thymine	Uracil
I*	Butanol	86	0.36	0.16	0.20	0.52	0.38
	Water	14					
II	Butanol	65	0.38	0.25	0.31	0.60	0.46
	99% Ethanol	15					
	80% Formic acid	7					
	Water	13					
III*	Butanol	77	0.18	0.0	0.16	0.51	0.42
	Formic acid	10					
	Water	13					
IV†	Butanol sat. w. water at 14°C		0.29	0.11	0.17	0.56	0.46

* Markham & Smith[5]. † Vischer & Chargaff[1]; Hotckiss[2].

dissolve purines according to Wyatt[4] and 0.01 *N* HCl to dissolve pyrimidines according to Carter.[8]

5) Measurement of the extinctions. The absorption intensity may sometimes undergo change due to the contamination of the paper or the impurity in the quartz of which the cell is made, so that accurate determination is accomplished only when the differences between two extinctions, one the maximum and the other an appropriately chosen, are adopted for the calculation of the amounts of the bases, as Vischer & Chargaff[9] recommended.

The writers picked out as the arbitrary extinctions those at the wavelength of 290 mμ for adenine, thymine and uracil and those at the wavelength of 300 mμ for guanine and cytosine, in compliance with their characteristic absorption curves. The differences between the respective two extinctions (ΔE) which were measured with the standard solutions containing each 10 γ of a base per cc. (Regarding the samples of the bases taken, see below) were as follow.

Base	Solvent	ΔE
Adenine	0.1 *N* HCl	$E_{262.5}-E_{290}=0.919$
Guanine	"	$E_{249}-E_{300}=0.705$
Cytosine	0.01 *N* HCl	$E_{275}-E_{300}=0.943$
Thymine	"	$E_{265}-E_{290}=0.556$
Uracil	"	$E_{258}-E_{290}=0.740$

The extinctions 1.0 and less of all the bases followed exactly Lambert-Beer law.

6) Absorption by the contamination of the paper. At first, a sliver of Toyo Roshi filter paper No. 3 was irrigated with Wyatt solvent without prior sampling of the bases, and various portions of the sliver were extracted with 5 cc. of 0.01 *N* HCl. The absorptions of the solutions varied according to the size of the paper portions as shown in Table II, all delineating a very slow spectral curve with a low maximum at λ 255 mμ (Cf. Fig. 1). Judging from the findings, such differences in size between the paper portions as are cut out for the control run of the analysis do not cause erroneous result beyond tolerable limit.

TABLE II

Ultraviolet Absorption of the Solutions after Extraction with 0.01 *N* HCl of Portions of Toyo Roshi Filter Paper No. 3, Irrigated with Wyatt Solvent without Prior Sampling of the Bases

Size of paper portions	Extinction		
	E_{240}	E_{255}	E_{300}
2 cm. × 2 cm.	0.028	0.030	0.006
3 cm. × 3 cm.	0.057	0.067	0.016
4 cm. × 4 cm.	0.093	0.109	0.036
5 cm. × 5 cm.	0.146	0.177	0.055

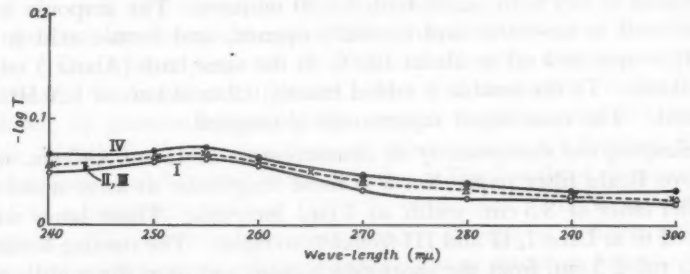

Fig. 1. Ultraviolet absorption spectra of the solutions obtained by extraction of various portions of a Toyo Roshi filter paper No. 3 strip which had been irrigated with Wyatt solvent without prior sampling.
I, II, III: 3.5 cm. × 3 cm. squares were extracted with 5 cc. of 0.01 *N* HCl at 37°C for 24 hours. IV: a 3.5 cm. × 2.5 cm. square was extracted with the same volume of 0.1 *N* HCl under the same conditions as above.

Next, paper portions 4 cm. × 2 cm. square at distances of 0, 10, 20 and 30 cm. from the starting line were extracted also with 5 cc. of 0.01 *N* HCl. Here E_{255} values measured 0.064, 0.063, 0.064 and 0.065, and E_{300} values 0.021, 0.020, 0.022 and 0.020 respectively without noticeable dis-

crepancy from one another in each set, proving that it is not absolutely necessary to use the portions of Lane III (S. below) just at the heights corresponding to the base spots for the control run.

Furthermore, the appropriate concentration of HCl in the solvent for extraction must be selected. When 5 cm. × 3 cm. portions of a paper unspotted and irrigated were extracted with 5 cc. of 0.01, 0.1 and 1.0 *N* HCl, the solutions showed E_{255} values of 0.185, 0.210 and 0.265 respectively, extinction increasing in parallel with the HCl normality. Vischer & Chargaff[1] used 1 *N* and 0.1 *N* HCl for extraction of guanine and adenine respectively and Wyatt[4] 0.1 *N* HCl for extraction of purines as well as pyrimidines. The writers employ 0.1 *N* HCl for purines and 0.01 *N* HCl for pyrimidines to evade most possibly the deleterious effect of HCl without lowering the readiness of extraction.

The Technique

The procedure will be described in full for the sake of readers' convenience, although the most part of it is no more than a collection of predecessors' inventions.

Hydrolysis. 5 to 8 mg. of a DNA are accurately weighed in a small hard glass tube (10 mm. × 100 mm.), and after addition of 0.2–0.3 cc. of formic acid with boiling point of 100–103°C,[10] sealed and heated in boiling water at first under shaking to change it into a fine suspension and afterwards at 175°C in an oil bath for 30 minutes. The ampoule is then cooled well in ice-water and carefully opened, and formic acid is completely evaporated off at about 160°C in the same bath (About 5 minutes are taken). To the residue is added exactly 0.2 or 0.3 cc. of 1 *N* HCl and agitated. The centrifuged supernatant is sampled.

Sampling and development of the chromatogram. A 15 cm. × 45 cm. square of Toyo Roshi filter paper No. 3 is lined lengthwise so as to make three parallel lanes of 3.5 cm. width at 1 cm. intervals. These lanes will be referred to as Lane I, II and III from left to right. The starting horizontal line is ruled 5 cm. from the short side bottom and onto the middle points of its sections belonging to Lane I and II (starting points) are sampled accurately 0.02–0.03 cc. and 0.01–0.02 cc. respectively of the clear solution above under slight heating over an electric heater. The paper sheet is hung precisely vertically on a horizontal glass rod into Wyatt solvent (This must be freshly prepared) filled in a glass dish of 16 cm. diameter so that the bottom of the paper may dip 1 cm. deep, and covered with a glass bell of 20 cm. diameter and 50 cm. height. 30 hours are requisite for the solvent to ascend 35 cm. at 26°C. After irrigation, the paper is dried within 1 hour by fanning over a heater. Then, Lanes I–III are cut off

and the first lane is placed for about 15 minutes in a bell room, wherein a small beaker containing concentrated ammonia is stood, in order to neutralize HCl remaining on the paper.

Detection of spots. Lane I is tightened on a wooden frame, sprayed with 0.005% fluorescein in 0.5 *N* ammonia, and exposed in a dark chamber to the ultraviolet rays, emitted from a Matsuda "sterilization lamp GL-15" (100 v., a.c., 15 w.) and filtered through a solution containing 35 g. of $NiSO_4 \cdot 7H_2O$ per 100 cc. in a 1.7 cm. (inner diameter) $\times$ 3 cm. glass tube whose both cut ends are cemented with quartz plates. The spots detected are margined in pencil and the paper strip is dried. Then, Lane II is lined as corresponds to the contours of the spots on Lane I and divided into portions 3–4 cm. long, each including a spot. From Lane III are also portions corresponding to those of Lane II are cut off for the control run.

It must be remarked here that the colored portion near the solvent front absorbs much light and spoils the spectrometric result, if cut even in part together with the neighbourhood for extraction.

Extraction. The portions of Lane II are separately sheared into small pieces and stood in contact with 5 cc. of 0.1 *N* HCl (for purines) or 0.01 *N* HCl (for pyrimidines) in stoppered (ground joint) 1.5 $\times$ 10 cm. tubes for 18 hours at 37°C. The portions of Lane III corresponding to those of Lane II are also treated in a similar manner.

Measurement of ultraviolet absorptions. The mixtures in the extraction tubes are each well stirred up and centrifuged. And the supernatants in the main run are examined against the control solutions in a spectrophotometer. The writers prepared the standard solutions with base specimens 1) adenine hydrochloride (crystal water 1/2 molecule) Zellstoff-fabrik Waldhof, 2) guanine hydrochloride (crystal water 1 molecule) Zellstoff-fabrik Waldhof, 3) cytosine General Biochemicals, 4) thymine General Biochemicals, and 5) uracil Wako Seiyaku. The last three specimens were dried to a constant weight at 110°C in vacuo and their N contents were analyzed. The wave-lengths of maximum absorption and the molecular extinction coefficients (ε) of the substances were found as follows:

Base	λ of max. abs. in mμ	$\varepsilon \times 10^{-3}$
Adenine	262.5	12.8
Guanine	249	11.4
Cytosine	275	10.44
Thymine	265	7.95
Uracil	258	8.29

The ultraviolet spectra are plotted in Fig. 2. ΔE values between the extinctions at maximum absorption and those at 290 or 300 mμ by 10 γ/cc solutions of the bases have been tabled above.

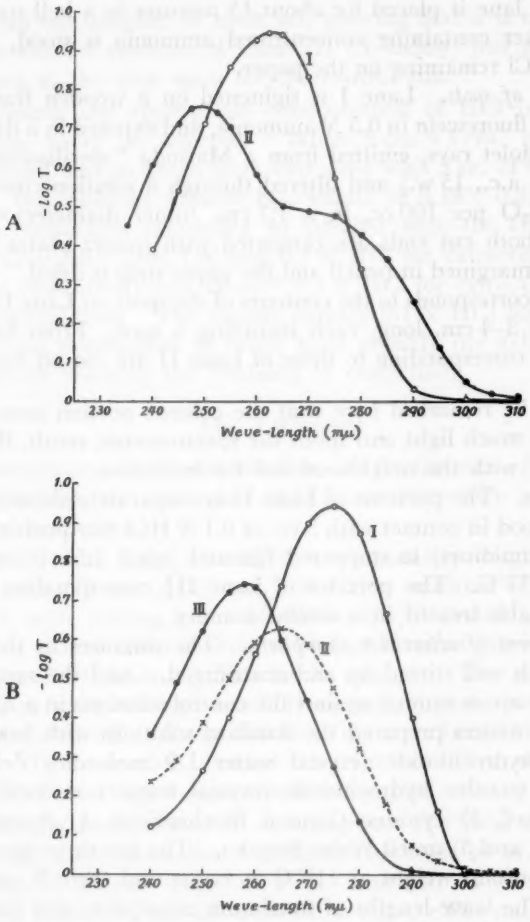

Fig. 2 (A, B). Spectra of purines and pyrimidines. A : those of 1 mg.% solutions of adenine (I) and guanine (II) in 0.1 *N* HCl. B : those of 1 mg.% solutions of cytosine (I), thymine (II) and uracil (III) in 0.01 *N* HCl.

Calculation. ΔE values of the bases between the respective two wavelengths above constitute regarding each of the bases a straight line function of the concentration in so far as the extinctions of 1.0 and less are concerned. The amounts of the bases in the solutions are read from the respective standard curves, and their percentages in the material are calculated out therefrom.

Remarks

1) The extinctions of 10 γ/cc solutions as well as the molecular extinction coefficients of the bases obtained by former investigators and the writers are compared in Table III.

TABLE III

Extinctions of 10 γ/cc Solutions and Molecular Extinction Coefficients ε (in parentheses) of the Bases Obtained by Different Authors

Base	Wave-length (mμ) whereat the extinction was measured	Author				
		Wyatt[4]	Vischer & Chargaff[7]	Hotchkiss[2]	Plossert[11]	The writers
Adenine*	260	0.96 (13,000)		1.01 (13,640)		
	262.5 (max. abs.)		0.93 (12,560)			0.947 (12,800)
Guanine*	250	0.73 (11,000)		0.655 (10,050)		
	249 (max. abs.)		0.737 (11,130)			0.758 (11,400)
Cytosine†	275 (max. abs.)	0.95 (10,500)		0.91 (10,110)	0.913 (10,140)	0.943 (10,440)
Thymine†	265 (max. abs.)	0.63 (7,950)		0.61 (7,690)		0.630 (7,950)
Uracil†	260	0.705 (7,900)		0.72 (8,070)		
	258 (max. abs.)				0.725 (8,130)	0.740 (8,290)

* Dissolved in 0.1 *N* HCl. † Dissolved in 0.1 *N* HCl excepting by Plossert who used 0.01 *N* HCl for cytosine and uracil (6th column).

2) Recovery. An example will be givin in detail:—5.1 mg. of a rat liver DNA specimen[13] were hydrolyzed and after evaporating off of formic acid, dissolved in 0.25 cc. of 1 *N* HCl, and 0.016 cc. of the centrifuged supernatant was subjected to paper chromatography. The solutions of adenine, guanine, cytosine and thymine obtained after extraction with 5 cc. of 0.1 *N* HCl each showed respective extinctions at wave-lengths of 260 and 300 mμ of 0.535, 0.006; 0.280, 0.040; 0.215, 0.022; and 0.310, 0.0; namely the total sum of E_{260}–E_{300} values reached 1.272. On the other hand, another 0.016 cc. of the centrifuged supernatant above was diluted up to 20 cc. with 0.1 *N* HCl, and examined as to E_{260} and E_{300}. The extinctions were 0.370 and 0.028 respectively, that is, E_{260}–E_{300} was 0.342. The solution subjected here to spectrometry was four times as dilute as the former solutions with respect to individual bases. Hence ΔE of this base mixture corresponding to the total sum of E_{260}–E_{300} values of the solutions of the individual bases is calculated at 1.368, and the recovery at 93%. In specimens of thymus- and spleen-DNA of rat[13] the recoveries reached

also 93%. The recoveries thus calculated do not represent those after the whole course of analysis, nevertheless, the main stages where errors are prone to occur have been scrutinized.

Recovery of the bases is often expressed in per cent of total N and P of the nucleic acid accounted for by the total sum of them, although it is possible not to give so accurate values as the ones above owing to analysis with separately weighed portions of a nucleic acid sample. Recoveries calculated in this way in calf thymus DNA[13] are shown in Table IV.

TABLE IV

Composition of a Calf-thymus DNA Preparation (analyzed by the writers)

Analysis	Content in per cent	Recovery (% of total) with respect to		Molecular ratio of bases to a total of 4
		N	P	
Adenine	8.89	37.50	27.96	1.23
Guanine	6.88	25.96	19.33	0.85
Cytosine	4.64	14.29	17.76	0.78
Thymine	7.70	13.91	25.88	1.14
Total bases	28.11	91.7	90.9	4
N*	12.3			
P†	7.2			
Ash‡	24.8			

* Micro Kjeldahl. † Modified Plimmer. ‡ Pregl.

3) The bases which have been extracted at 37°C are not readsorbed to the paper pieces on cooling. As regards this point, 0.01 cc. portions of a watery solution containing 2.1 mg. of thymine per cc. were put on a paper sliver, which had been irrigated with Wyatt solvent as above and dried, and after drying again, the paper portions of various sizes including each a thymine spot were extracted with 5 cc. of 0.01 *N* HCl in the manner also described. The mixtures of the solution and paper pieces were then placed in a refrigerator overnight to measure followingly the extinctions of the centrifuged supernatants. As shown in Table V, the base was found quantitatively recovered, regardless of the amount of the paper pieces with which the solution had been stood in contact at 0°C. The result after experimentation with adenine was the same.

4) According to Wyatt the bases of a DNA preparation contaminated with a PNA can be analyzed, if the preparation is previously fractioned by the method of Schmidt & Thanhauser[14]. The description could not be realized as will be demonstrated. 300 mg. of a commercial sperm DNA

TABLE V

Recovery of 21 γ Portions of Thymine, when the Solutions after Extraction of the Spots were Cooled prior to Separating off of Paper Pieces

Size of paper portions	E_{265}	E_{290}	$\Delta E(E_{265}-E_{290})$	Recovery	
				γ	%
2 cm.×2 cm.	0.270	0.036	0.234	21.06	100
3 cm.×3 cm.	0.270	0.040	0.230	20.70	99
4 cm.×4 cm.	0.287	0.053	0.234	21.06	100
5 cm.×5 cm.	0.270	0.035	0.235	21.15	101

specimen (Nutritionl Biochemicals Corporation), which was Molisch-positive and whose hydrolysate gave an uracil spot on a chromatogram, were dissolved in 15 cc. of 1 *N* NaOH and after standing at 37°C for 1 day, precipitated with 1.5 volumes of abs. alcohol. The deposit was dissolved in water, dialysed against changes of distilled water and precipitated with 2 volumes of abs. alcohol, followed by washing with alcohol and ether. The substance, dried in a vacuum-desiccator ($CaCl_2$) to a constant weight, contained 7.56% P and analyzed : adenine 6.88%, guanine 8.41%, cytosine 2.66%, thymine 7.14%, uracil 2.39%. Therefore, the PNA could not be perfectly removed. Elimination of PNA may be difficult for an old preparation, nevertheless, the result indicates at least that Schmidt and Thanhauser method is not always applicable.

Discussion

Predecessors' analyses of calf thymus DNA are given together with the present writers' in Table VI. The molecular ratios of the bases vary according to the author or the group of authors, although adenine and thymine amount more than guanine and cytosine in any set of analyses, that of Chargaff *et al.* on Prep. III excluded. The divergency of the ratios adenine/guanine, thymine/cytosine and purines/pyrimidines occurs by change of hydrolysis conditions as Wyatt[4] demonstrated, but those ratios are also changed by the action of DNA-ase during preparation of the nucleic acid as Chargaff[19] mentioned. Hence, the incoincidence of the analytical results in the table are not all ascribed to the difference of analytical methods themselves.

Former techniques at the individual stages have been criticized above, and so two supplementary notes only will be added here. To discuss at first as to Wyatt formic acid method of hydrolysis, the thymine value will lower, if the hydrolysis is incomplete, and on the other hand, if excessive

TABLE VI

Bases of Calf Thymus DNA in Molecular Ratios (analyses of various authors)

	Authors						
	Wyatt[4]	Chargaff *et al.*[15]			Chargaff & Lipshitz[16] (Average values of many preparations)	Woodhouse[18]	The writers
		Prep. I	Prep. II	Prep. III			
Adenine	1.11	1.15	1.06	1.14	1.37	1.00	1.23
Guanine	0.84	0.76	0.83	0.95	1.00	0.85	0.85
Cytosine	0.86	0.66	0.66	0.72	1.00	0.71	0.78
Thymine	1.13	1.03	1.00	0.94	1.34	0.99	1.14
5-Methylcytosine	0.056						
Total bases	4.00	3.60	3.55	3.75	4.71	3.55	4.00
Adenine/guanine	1.32	1.51	1.28	1.20	1.37	1.18	1.45
Thymine/cytosine	1.31	1.56	1.52	1.31	1.34	1.39	1.46
Purines/pyrimidines	0.98	1.12	1.16	1.26	1.01	1.09	1.08
Recovery*							
N	95	92	88	92			92
P	90	90	88	94	93.3		91
Hydrolysis agent, temp. and duration	98% formic acid, 175°C, 30 min.	Purines: 1 *N* H_2SO_4, 100°C, 1 hr. Pyrimidines: 98-100% formic acid, 175°C, 2 hrs.			98-100% formic acid, 175°C, 2 hrs.[17]	72% (w/v) or 11.1 *N* $HClO_4$, 100°C, 1 hr.	More than 98% formic acid, 175°C, 30 min.

* % of total accounted for by estimated bases.

decomposition takes place, the purines/pyrimidines ratio will decrease due to the break up of purines which are quickly liberated as compared with pyrimidines. The writers' findings did not suggest such inadequacies. Next, in the present procedure of analysis, the spots in the first lane which are marked is not applied to extraction and the areas in the second lane corresponding to those spots are possible not to accurately cover the real spots. But the solutions after extraction of the paper portions cut out with precautions proved sufficient recovery even with respect to thymine which has the largest Rf value and is most diffused and most subject to dislocation.

SUMMARY

1. Wyatt method for determination of the bases in DNAs was modified by combining Vischer & Chargaff's and Makino & Matsuzaki's with simplifications.

2. The bases can be estimated by this combination with recovery of more than 90%.

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H. Masamune.

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Biochemical Studies on Carbohydrates

CLXXVI. Analysis of Mutarotation of N-Glycosides

Second Paper

On Aniline-N-riboside

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In 1946, Berger & Lee[1] obtained two isomers of aniline-N-riboside, one with F.P. of 137–9° and $[\alpha]_D^{w}$ in pyridine of +177° by refluxing the components with ethanol and the other with F.P. of 125–7° and $[\alpha]_D^{w}$ in pyridine of +63.4° by allowing the components react in aqueous ethanol at room temperature. In pyridine the isomers mutarotated but did in the same direction to different end values, the specific rotation of the more dextrorotatory arriving at +156.5° and that of the less dextrorotatory at +48.6°. Since the rotation change did not appear to be of $\alpha\beta$ type, the authors made structural study of the substances by acetylation and tritylation, and concluded the former to be aniline-N-α-ribofuranoside and the latter to be aniline-N-α-ribopyranoside. However, the isomers prepared by Howard *et al.*[2] (Isomer I: F.P. 126–7°, $[\alpha]_D$ in pyridine +180°; Isomer II: F.P. 119°, $[\alpha]_D$ in pyridine +60°) did not mutarotate in pyridine and gave only identical acetyl derivatives aniline-N-ribopyranoside-2:3:4-triacetate. Ellis & Honeyman[3] found that their preparations (Isomer I: F.P. 133–4°, $[\alpha]_D$ in pyridine +176.4°; Isomer II: F.P. 112–4°, $[\alpha]_D$ in pyridine +60.2°) did not mutarotate in pyridine even after addition of a drop of water, but showed rotation change in methanol and that in the same direction and to approximately the same end-point, in which they conjectured involvement of production of a third compound. Similar mutarotation not only in methanol but also in pyridine of the isomers (Isomer I: F.P. 123–4°; Isomer II: F.P. 114–6° (was observed by Butler *et al.*[4]

The writer made clear in the foregoing article[5] of this series of investigations that one of the two isomers of aniline-N-lactoside, which had been prepared by heating the components with dry methanol for a long time and was less levorotatory, was α-lactoside, and the other given by

condensation of the components in the presence of water was β-lactoside, and further could obtain the latter by the procedure of Berger & Lee for preparing the less dextrorotatory riboside (unpublished). That the two isomers of the riboside and those of the lactoside are prepared in similar manners made the writer surmise that the former pair of isomers are also anomers identical with respect to the lactol ring.

Preparation procedure. The writer followed strictly the procedures of Berger & Lee to prepare the isomers of the riboside. The more dextrorotatory (Isomer **I**) given by heating the components in dry ethanol melted at 130–2° and the less dextrorotatory (Isomer **II**), synthesized in aqueous ethanol, at 124–6°. Ellis & Honeyman said that which of the isomers is given depends on presence or absence of water in the condensation medium. Nevertheless, the writer obtained Isomer **II** in stead of Isomer **I** in some of the attempts where water was completely avoided. In these cases, the products changed into Isomer **I** on refluxing again with their mother fluids, but on the other hand, Isomer **II** gradually crystallized out always when a mother fluid of Isomer **I** was stood in a refrigerator after stoppering tightly. In general, Isomer **I** appears to form in a water-free reaction mixture of enough concentration as totally solidifies in an instant on cooling. It is probably due to the same mechanism that aniline-N-α-lactoside is given when the reaction mixture is carried down quickly with a large excess of ether, although this operation gave rise to no production of Isomer **I** of the riboside which is comparatively readily soluble in an alcohol-ether mixture. From what has been described, the more dextrorotatory isomer of the riboside looks to be the labile form. In fact, it became the less dextrorotatory when exposed to air for 2 days getting colored light brown, and even when stored in vacuo, though slowly.

Mutarotation in methanol. In methanol the specific rotation of Isomer **I** of the riboside changed at 5°C from +131.2° to +11.0° in about 15 hours and that of Isomer **II** from +26.5° to +8.7° after about the same duration of time. Thus the end values nearly coincided with each other, although the mutarotations proceeded in the same direction. To dispose mathematically, log $(r_t - r_\infty)$ values of Isomer **I** were plotted against time t at first (r specific rotation). As shown in Table I and Figs. 1 & 2, the mutarotation of this isomer is caused by two reactions, the first quick (Reaction I) and the second slow (Reaction II). The velocity constant of Reaction II (K_{II}) can be calculated out from the rotation values in the long period, wherein the rotations of the both isomers are the same, that is, when they are at equilibrium between themselves, by the following equation:

TABLE I

Mutarotation of Isomer **I** of Aniline-N-riboside in Dry Methanol

c 3.0, temp. 5°C, rotation tube 1 dm.

Time after dissolution	$[\alpha]_D^5$ (°)	K_{II}	K_I
0 min.	+131.2*		
8	+118.7		0.0048
18	+106.5		0.0043
30	+ 80.7		0.0065
41 1/2	+ 70.4		0.0060
50 1/2	+ 60.5		0.0062
1 2/60 hrs.	+ 51.1		0.0061
1 26/60	+ 39.3		0.0058
2 18/60	+ 24.9		Avg.: 0.0059
3	+ 19.6		
3 38/60	+ 18.5		
4 26/60	+ 16.7		
6	+ 15.0	0.0016	
6 22/60	+ 14.7	0.0016	
7 16/60	+ 14.0	0.0016	
8 30/60	+ 13.6	0.0014	
11 18/60	+ 12.4	0.0015	
12 30/60	+ 11.7	0.0015	
23 40/60	+ 11.0	Avg.: 0.0015	

* Calculated from the intercept on the ordinate of the curve of log $(r_t - r_\infty)$ of Isomer **I** in Fig. 2.

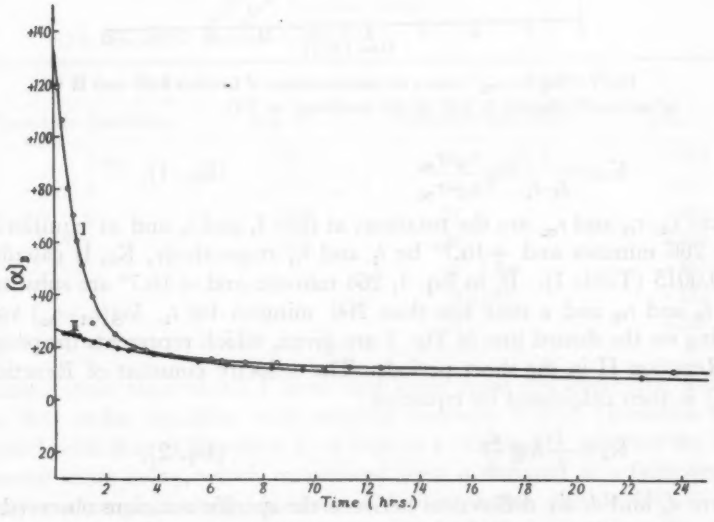

Fig. 1. Mutarotation of Isomers **I** (I) and **II** (II) of aniline-N-riboside (c 3.0) in dry methanol at 5°C.

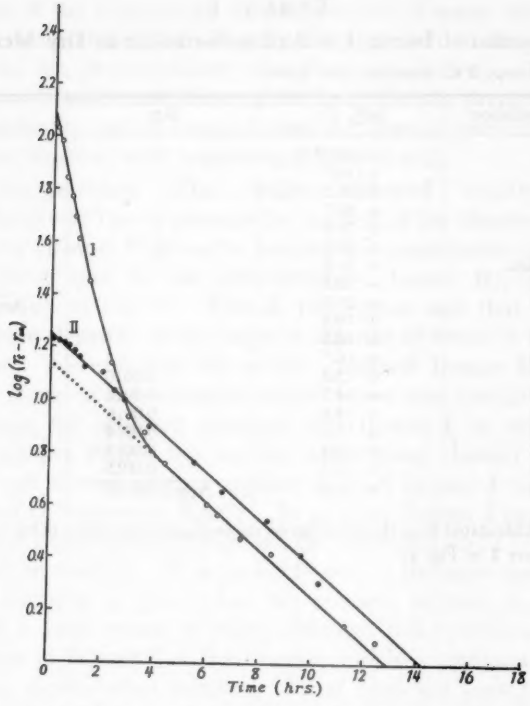

Fig. 2. log $(r_t - r_\infty)$ values in mutarotations of Isomers **I** (I) and **II** (II) of aniline-N-riboside (c 3.0) in dry methanol at 5°C.

$$K_{II} = \frac{1}{t_2 - t_1} \log \frac{r_{t1} - r_\infty}{r_{t2} - r_\infty} \qquad \text{(Eq. 1)},$$

where r_{t1}, r_{t2} and r_∞ are the rotations at time t_1 and t_2 and at equilibrium. Let 266 minutes and $+16.7°$ be t_1 and r_{t1} respectively, K_{II} is calculated at 0.0015 (Table I). If, in Eq. 1, 266 minutes and $+16.7°$ are substituted for t_2 and r_{t2} and a time less than 266 minutes for t_1, $\log(r_{t1} - r_\infty)$ values falling on the dotted line in Fig. 2 are given, which represents the progress of Reaction II in the short period. The velocity constant of Reaction I (K_I) is then calculated by equation:

$$K_I = \frac{1}{t} \log \frac{d_o}{d_t} \qquad \text{(Eq. 2)},$$

where d_o and d_t are differences between the specific rotations observed and the corresponding rotations on the dotted line at t_o and t. Reckoned thus, it was found 0.0059, which is fourfold as large as K_{II}. As to Isomer **II**, the

TABLE II

Mutarotation of Isomer II of Aniline-N-riboside in Dry Methanol

c 3.0, temp. 5°C, rotation tube 1 dm.

Time after dissolution	$[\alpha]_D^5$ (°)	K_{II}	
0 min.	+26.5*		
8	+25.4		
20	+25.4	0.0010	
32	+25.2	0.0010	
41	+24.7	0.0011	
54 1/2	+24.1	0.0011	
1 5/60 hrs.	+23.1	0.0014	
1 32/60	+22.0	0.0014	
2 1/60	+21.0	0.0013	
2 24/60	+19.5	0.0015	
2 50/60	+18.7	0.0015	
3 46/60	+16.6	0.0016	Avg.: 0.0015
5 30/60	+14.5	0.0015	
6 35/60	+13.2	0.0015	
8 18/60	+12.2	0.0016	
9 36/60	+11.3	0.0015	
10 17/60	+11.1	0.0015	
22 30/60	+ 8.7	Avg.: 0.0014	

* Calc. as in Table I.

TABLE III

Mutarotation of Isomers I and II of Aniline-N-riboside in Abs. Ethanol

c 3.0, temp. 5°C, rotation tube 1 dm.

Isomer I		Isomer II	
Time after dissolution	$[\alpha]_D^5$ (°)	Time after dissolution	$[\alpha]_D^5$ (°)
1 hr.	+139.0	10 min.	+22.8
4	+117.3	14 1/2	+25.7
6	+106.9	27	+25.9
12	+ 86.6	30	+26.3
		2 hrs.	+26.9
		3 15/60	+25.8
		12	+23.1

mutarotation after about 1 hour and more from the beginning followed the first order equation with velocity constant 0.0015 (Reaction II) in accord with that of Reaction II of Isomer I (Table II), whereas the initial showed some delay, which manifested itself in ethanol as a faint opposite rotation change, namely, increase of dextrorotation (Table III).

Now that the mutarotation curves of the both isomers joined together far before arriving at the end value, it is beyond doubt that Reaction I is

anomerization and Reaction II production of a third compound from the equilibrated mixture of the two isomers. In other words, Isomer **I** must be aniline-N-α-riboside and Isomer **II** aniline-N-β-riboside. In the anomerization (Reaction I) the velocity of $\beta \rightarrow \alpha$ reaction is very small in comparison with that of $\alpha \rightarrow \beta$ change or even negligible, and therefore the equilibrated mixture is considered to comprise almost solely the β-anomer. Regarding the product of Reaction II will be made a contemplation below. To add, no hydrolysis of the riboside occurred in methanol at all, as will be detailed also below. The writer assigns pyranoid structure to the isomers like to free sugars, because no reliable means is conceivable nowadays to establish the ring structure as Honeyman & Tatchell already mentioned.[5a]

TABLE IV

Mutarotation of Isomers **I** and **II** of Aniline-N-riboside in Various Watery Buffer Solutions

c 1.0, temp. 5°C, rotation tube 1 dm.

Isomer I		Isomer II	
Time after dissolution	$[\alpha]_D^5$ (°)	Time after dissolution	$[\alpha]_D^5$ (°)
At pH 5.6 (*M*/5 acetate buffer)			
12 1/2 min.	− 9.8	10 min.	−10.3
15	−10.6	13	−11.4
30	−11.0	36	−13.0
1 4/60 hrs.	−11.8	1 30/60 hrs.	−13.4
2 9/60	−11.7	2 45/60	−13.4
3 30/60	−12.3	8	−13.8
5	−12.5		
8	−12.4		
At pH 7.0 (*M*/15 phosphate buffer)			
10 1/2 min.	+55.0	15 1/2 min.	+ 5.8
15	+51.6	36	+ 2.2
30	+42.8	1 18/60 hrs.	− 2.2
48	+32.6	1 48/60	− 4.5
61	+24.8	2 30/60	− 5.8
1 15/60 hrs.	+19.1	4 15/60	− 8.6
1 50/60	+ 7.5	5 21/60	− 9.2
3	0.0	8 19/60	−10.2
3 39/60	− 3.9		
4 54/60	− 6.6		
7 9/60	− 7.6		
At pH 9.6 (*M*/10 veronal sodium salt-*N*/10 HCl)			
24 min.	+62.8	30 min.	+10.4
45	+62.6	1 24/60 hrs.	+11.4
6 30/60 hrs.	+63.0	4 30/60	+12.3
12	+62.1	12 45/60	+11.6

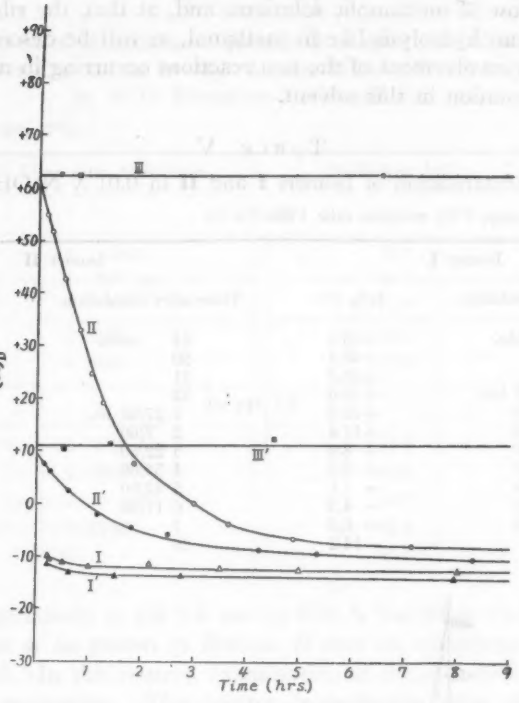

Fig. 3. Mutarotation of the two isomers of aniline-N-riboside (c 1.0) in various watery buffer solutions at 5°C. I, I′: Isomers **I** and **II** respectively in acetate buffer pH 5.6; II, II′: Isomers **I** and **II** respectively in phosphate buffer pH 7.0; III, III′: Isomers **I** and **II** respectively in veronal buffer pH 9.6.

Mutarotation and hydrolysis in water at various pH's. The courses of rotation changes at 5°C in *M*/5 acetate buffer pH 5.6, *M*/15 phosphate buffer pH 7.0, veronal buffer (*M*/10 veronal sodium salt-*N*/10 HCl) pH 9.6 and 0.01 *N* NaOH (pH of the solution about 12.5) are illustrated in Tables IV and V and Figs. 3 and 4. At pH 5.6, the specific rotations of the both isomers decreased instantly (in 10 minutes or so) to about −10° and after further slight decrease, remained constant at about −13° which approximates to the specific rotation of free ribose (−15.3°). At pH 7.0 the change delineated slower curves, and at pH 9.6 no significant alteration of rotation took place at least within first 12 hours, while on the contrary noticeable mutarotation occurred again at about pH 12.5 (in 0.01 *N* NaOH). The mutarotation curves in the last case (Table V & Fig. 4)

resembled those of methanolic solutions, and, at that, the riboside underwent almost no hydrolysis like in methanol, as will be described. These facts indicate involvement of the two reactions occurring in methanol also in the mutarotation in this solvent.

TABLE V

Mutarotation of Isomers **I** and **II** in 0.01 *N* NaOH

c 1.0, temp. 5°C, rotation tube 1 dm.

Isomer **I**		Isomer **II**	
Time after dissolution	$[\alpha]_D^5$ (°)	Time after dissolution	$[\alpha]_D^5$ (°)
14 min.	+53.4	24 min.	+10.0
23	+46.3	30	+10.7
46	+39.7	41	+ 9.1
1 12/60 hrs.	+35.6	52	+ 8.2
1 48/60	+19.4	1 27/60 hrs.	+ 6.0
2 38/60	+11.4	2 7/60	+ 5.0
3 24/60	+ 8.0	3 22/60	+ 2.5
4 30/60	+ 6.6	4 53/60	− 0.9
7 15/60	− 1.1	5 42/60	− 3.6
8 5/60	− 4.3	6 17/60	− 3.9
9 50/60	− 6.6	7	− 5.0
26	−14.2	26	−14.7

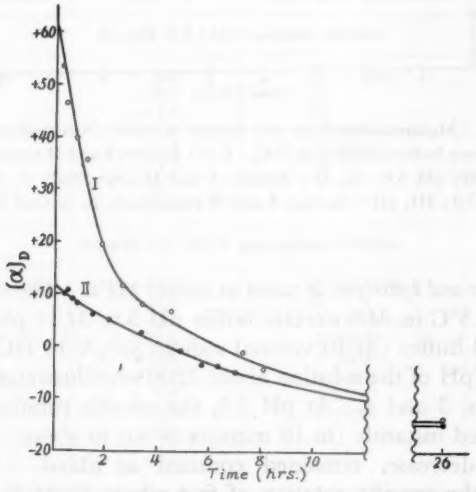

Fig. 4. Mutarotation of Isomers **I** (I) and **II** (II) of aniline-N-riboside (c 1.0) in 0.01 *N* NaOH at 5°C.

Hydrolysis of the riboside proceeded quickly at pH 5.6, reaching 83% in 3 hours, and less quickly at pH 7.0 (Table VI), but it reached only 3.8

TABLE VI

Progress of Hydrolysis of Isomer **II** of Aniline-N-riboside after Dissclution in *M*/5 Acetate Buffer pH 5.6 and in *M*/15 Phosphate Buffer pH 7.0

c 2.0, temp. 5°C.

Time after dissolution	Hydrolysis (%)
at pH 5.6	
18 min.	26.1
1 3/60 hrs.	51.4
2	72.7
3 8/60	83.2
4 30/60	82.6
at pH 7.0	
1 2/60 hrs.	14.2
2 15/60	30.8
4	42.3
7 30/60	61.4
10	61.0

and 1.8% respectively at pH 9.6 and in 0.01 *N* NaOH in 24 hours.

The nature of the product by Reaction II from the riboside in methanol and 0.01 *N NaOH.* In this respect, examination of the ultraviolet absorption afforded no suggestion. The spectra immediately after dissolution of Isomer **I** in these solvents were essentially identical with that of aniline-N lactoside, showing maxima at wave-lengths of 240 or 237.5 mμ and 287.5 or 285 mμ and a minimum at the wave-length of 262.5 mμ, and they did not vary with lapse of time despite of mutarotation. Next, Pigman *et al.*[6] noticed that glycosylamines turned levorotatory in glacial acetic acid, following the first order equation, although they could not determine the end values due to gradual browning of the solutions. In the riboside solutions in 0.01 *N* NaOH the writer witnessed no browning at least with the naked eye so that the product occurring here, which is more levorotatory than Isomers **I** and **II**, looks to differ from that in glacial acetic acid. The writer also dissolved Isomer **II** of the riboside in glacial acetic acid and found increase of levorotation, though slight, (Table VII) and simultaneous development of a brown color. This solution exhibited strong increase of absorption over the wave-lengths 320–400 mμ with time in contrast to the solution in methanol (Fig. 5). Thirdly, Isbell & Frush[7] observed mutarotation of β-galactosylamine in water-containing ammonia in the same direction as the α-anomer. This anomalous mutarotation was as-

TABLE VII

Mutarotation of Isomer II of Aniline-N-riboside in Glacial Acetic Acid

c 1.0, temp. 15°C, rotation tube 1 dm.

Time after dissolution	$[\alpha]_D^{15}$ (°)
12 min.	+ 3.6
39	− 2.1
1 30/60 hrs.	− 7.5
2 45/60	− 9.7
3 20/60	−10.8

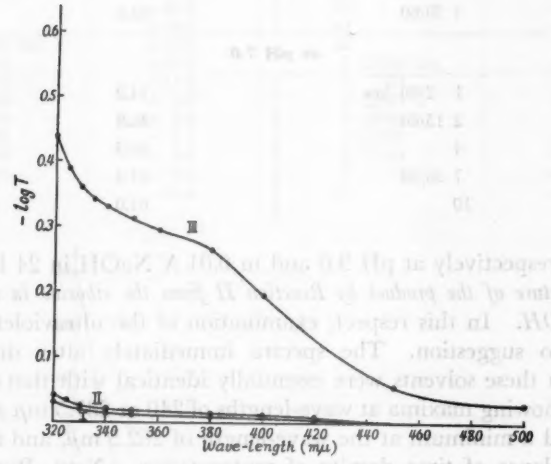

Fig. 5. Absorption spectra of solutions of Isomer II of aniline-N-riboside in near-ultraviolet region. I: A 3% methanolic solution was stood at 5°C and after various durations of time its portions were taken out, diluted to 1.43 mg/cc with methanol and examined spectrometrically. The spectra were the same at least within 24 hours after preparation of the original solution. II: A 1% solution in glacial acetic acid was prepared and a portion was immediately diluted with methanol to 1.43 mg/cc to measure the extinctions. III: Another portion of the solution in II was taken out after standing the solution at 15°C for 3 hours and diluted as above for the examination.

cribed by the authors not to establishment of $\alpha\beta$-equilibrium but to formation of probable digalactosylamine with specific rotation of +22°. In the writer's case, however, appearance of diribosylaniline, which accompanies liberation of aniline, is improbable, because, first, more than 2.1% of aniline could not be detected free even after 96 hours at 25°C in a me-

thanolic solution of the riboside (Isomer **II**) and 1.8% only could be after 24 hours in 0.01 *N* NaOH at 5°C, and, second, paper chromatographic analysis (Solvent water-sat. butanol-ethanol (9 : 1 by volume) mixture) of solutions in methanol and 0.01 *N* NaOH demonstrated a strong spot corresponding to aniline-N-riboside and a faint spot of free ribose but none else (See Experimental). Diribosylaniline should have an Rf value intermediate between those of aniline-N-riboside and ribose.

Finally, a polarographic study was attempted. Aniline-N-riboside is quantitatively recovered from its methanolic solution, stood long, by drying in vacuo, as will be described in the experimental part, and moreover, no change of ultraviolet absorption spectrum of it occurs neither in methanol nor in 0.01 *N* NaOH as noted already. These findings disclose that not scission but rearrangement in the molecule of the riboside is the cause of giving the unknown product. Cantor & Penington[8] found equilibrated, concentrated (0.25 *M*) solutions of various aldoses to be reduced at the dropping mercury cathode, and attributed it to aldehydo form of the sugars in the solutions, whose amount depended on the kind of sugar, varying with the mutarotation velocity, and was particularly abundant in ribose capable of very quick mutarotation. N-Glycosides mutarotate like free sugars so that accumulation of Schiff base corresponding to aldehydo form of free sugars is inferred particularly with respect to such N-glycosides as riboside. Not only acetone and aliphatic aldehydes as such but also ketones dissolved together with acid hydrazine or phenylhydrazine[9] or changed into Girard derivatives[10] are reduced at the dropping mercury cathode. Since this is certainly due to formation of the C=N bond, the writer made polarographic analysis of the riboside, employing a 0.031 *M* solution of Isomer **II** in 0.01 *N* NaOH containing 0.1 *M* LiCl. The solution showed a reduction wave with a half-wave potential of −1.26 volt (referred to the sat. calomel electrode) soon after preparation (Fig. 6A) and a more marked at the 24th hour (See Fig. 6B). In the solvent here, the riboside scarcely splits into aniline and ribose, and in fact, the reduction wave of free ribose whose half-wave potential in 0.01 *N* NaOH is −1.86 volt (Fig. 6E) did not appear. On the other hand, a 0.1 *M* aniline-N-lactoside solution in 0.01 *N* NaOH, in which the glycoside makes only $\alpha\beta$-isomerization[5], as well as a 0.031 *M* solution of Isomer **II** of the riboside in a carbonate buffer pH 9.6, which exhibits no mutarotation, gave no reduction wave (Figs. 6D and 6C). In short, the reduction wave mentioned of the riboside solution is given neither by α and β forms of the probable ribopyranoside nor by free ribose. Thus Reaction II accompanied by increase of levorotation might be believed to be formation of a Schiff base. Aniline-N-ribofuranoside would also show a reduction wave by the aldehydo form of ribose freed at the cathode, because furanoside is suspected

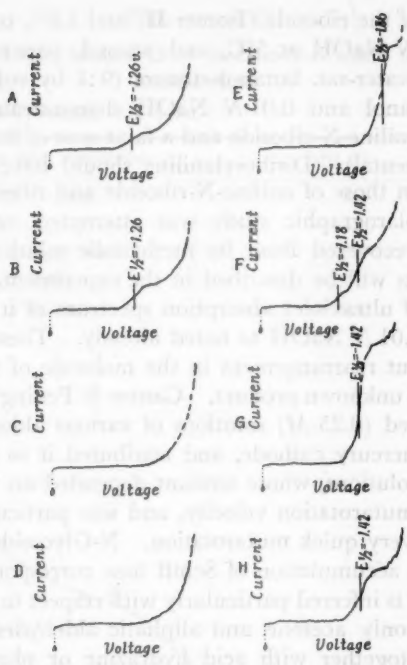

Fig. 6. Polarograms. A and B: A 0.031 *M* solution of Isomer **II** of aniline-N-riboside in 0.01 *N* NaOH was analyzed immediately (A) and 24 hours (B) after preparation, taking portions. C: A 0.031 *M* solution of Isomer **II** of aniline-N-riboside in *M*/10 carbonate buffer pH 9.6 was analyzed after 2 hours from preparation. D: A 0.1 *M* solution of aniline-N-β-lactoside in 0.01 *N* NaOH was analyzed immediately after preparation. E: 0.01 *M* D-ribose in 0.01 *N* NaOH was analyzed 30 minutes after preparation. F and G: 0.031 *M* aniline-N-riboside (Isomer **II**) in *M*/5 acetate buffer pH 5.6 was analyzed after 10 minutes from preparation of the solution (F) and after 10 hours (G) with its portions. H: 0.007 *M* ribose in *M*/5 acetate buffer pH 5.6 was examined after 20 minutes from preparation.

to be less stable than the pyranoside and the furanose which possesses stronger reduction power to be capable of giving more of the aldehydo form than the pyranose. But, as above remarked, the half-wave potential of the riboside solution was distinctly above that of a ribose solution.

Analysis of mutarotation of riboside solutions at pH 5.6 *and* 7.0. At pH 5.6, the rotations of both of the isomers were found to have changed nearly to the equilibrium value already at the first measurement, which can not be elucidated by mere hydrolysis amounting no more than 26% after 18 minutes from dissolution of the substance (analyzed in Isomer **II**). The

source of the enormous increase of levorotation was looked in Schiff base formation. As is shown in Fig. 6F, the solution of Isomer **II** at pH 5.6 gave two reduction waves with half-wave potentials −1.18 and −1.42 after 10 minutes from preparation. The wave with the lower potential is regarded as due to appearance of free ribose (Comp. with Fig. 6H) and the one with the higher potential most probably to the appearance of the Schiff base. The former wave was no more recognized after 10 hours of standing of the solution in contrast to the remarkable enlargement of the latter (Fig. 6G), so that it is assumed that the Schiff base once formed is replaced gradually by the free sugar after about 1 hour despite of constant rotation values. Since occurrence of anomerization at the very beginning is not excluded also here, the rotation change of the riboside at the present pH owes most probably to Schiff base formation, hydrolysis and almost neglectable anomerization.

The mutarotation at pH 7.0 will only represent slower progress of those reactions.

Experimental

*Preparation of Isomer **I** of aniline-N-riboside.* 0.3 g. of D-ribose and 0.2 cc. of aniline (redistilled) were refluxed with 3 cc. of abs. ethanol for 2 hours. Soon on cooling the reaction mixture changed into a solid mass, which was brought onto a suction filter, washed with ice-cooled abs. ethanol and dried in vacuo over $CaCl_2$. 0.3 g. (67% of theory) of hygroscopic, microscopic crystals were given. F.P. 130–2°. N (micro Kjeldahl) 6.19% (Calc. for $C_{11}O_4H_{15}N$ 6.22%). $[\alpha]_D^{\gamma}$ in pyridine +173.8° (c 1.5; no mutarotation). Its mixture with a specimen of Isomer **II** (F.P. 122–4°) melted at 126–7°, namely mixed melting point test showed no depression.

Remarks. 1) The filtrate from the crystals, tightly stoppered in a flask, was stood in a refrigerator, whereby crystals in large prisms came out slowly. It melted at 123–4° and had $[\alpha]_D^{18}$ in dry methanol of +24.8°→+11.8° (c 1.0) (Cf. the text), proving its identity with Isomer **II** below. Since the isomers show no depression of melting point when mixed, the test was abandoned.

2) 0.1 g. of Isomer **I** was stood in air for 2 days. The substance got tinged brownish and showed F.P. of 124° and $[\alpha]_D^{20}$ in dry methanol of +23.8°→+12.0° (c 1.2) (Cf. the text). This was again refluxed with 1 cc. of abs. ethanol for 2 hours. The crystals obtained here (0.05 g.) melted at 130–1° and had $[\alpha]_D^{18}$ in dry methanol of +127.6°→+11.7° (c 1.2) (Cf. the text).

*Preparation of Isomer **II**.* 0.3 g. of D-ribose was dissolved in 4 cc. of water and acidified with 0.1 *N* H_2SO_4 to pH 4.0, and 2 cc. of an ethanolic solution containing 0.2 cc. of aniline were added. The mixture was shaken for 10 minutes in water at 25°C and then stood in a refrigerator overnight. Large needles precipitated. The deposit was filtered off, washed with ice-cooled abs. ethanol and dried in vacuo over $CaCl_2$. Yield 0.4 g. (89% of theory). F.P. 124–6°. N 6.19% (Calc. 6.22%). $[\alpha]_D^{\gamma}$ in pyridine=+62.1° (c 1.5; no mutarotation).

Measurement of hydrolysis of the isomers I and II of aniline-N-riboside. It was effected as already reported in the preceding paper[5], treating the methanolic solutions of the riboside also just like the watery.

Recovery of Isomer II from a methanolic solution of Isomer I after standing. The methanolic solution of Isomer **I** (30 mg/cc), whose rotation change at 5°C had been followed up nearly till standstill, was dried in a vacuum desiccator ($CaCl_2$) at room temperature. The crystalline residue melted at 122–4° and had $[\alpha]_D^{18}$ in dry methanol of $+25.3° \rightarrow +12.1°$ (c 1.5) (Cf. the text).

Polarographic analysis. A Shimazu polarograph was employed. 0.031 *M* aniline-N-riboside in 0.01 *N* NaOH, *M*/10 carbonate buffer pH 9.6 and *M*/5 acetate buffer pH 5.6, 0.1 *M* aniline-N-β-lactoside and 0.01 *M* ribose in 0.01 *N* NaOH and 0.007 *M* ribose in *M*/5 acetate buffer pH 5.6 were analyzed immediately after preparation and after standing at 5°C, adding LiCl to 0.1 *M* as an indifferent electrolyte in advance to the measurement. Constants for capillary: m=0.00198 g. of mercury per second; t=3.25 average seconds per drop. S (sensitivity)=1/20. Temperature=20°C. Half-wave potentials were all referred to the saturated calomel electrode. Examination of the dry-methanolic solutions was given up because of faint electric conductivity of them.

Paper partition chromatography. 30 mg. of Isomer **I** of the riboside were dissolved in 1 cc. of dry methanol and 10 mg. of the same isomer in 1 cc. of 0.01 *N* NaOH. 0.02 cc. portions were pipetted out from the solutions immediately after preparation and after standing for 10 and 24 hours at 5°C, and sampled on strips of Toyo Roshi filter paper No. 2 (2.5×50 cm.) separately, taking two portions at one time from each of the solutions. And the strips were irrigated with a mixture of butanol, saturated with water, and ethanol (9:1 by volume) at 25°C as long as 30 cm. A pair of the chromatograms were sprayed one with the aniline hydrogen phthalate reagent of Partridge (for ribose) and the other with Ehrlich reagent (0.5 g. of *p*-dimethylaminobenzaldehyde in a mixture of 100 cc. of ethanol and 2 cc. of conc. H_2SO_4) (for aniline).

Summary

1. Two isomers of different $[\alpha]_D$ values (**I** and **II**) of aniline-N-riboside were prepared after Berger & Lee and their mutarotations in various solvents were investigated as to the reaction mechanisms to which they owe.

2. The mutarotations in methanol and 0.01 *N* NaOH are considered as due to rapid isomerization of $\alpha\beta$ type and slow Schiff base formation.

3. At pH 9.6 the riboside scarcely shows rotation change. Most probably no molecular rearrangement nor scission takes place.

4. Mutarotations of the riboside at pH 5.6 and 7.0 are combined results of anomerization, Schiff base formation and hydrolysis.

5. The more dextrorotatory isomer (**I**) is assumed as aniline-N-α-ribopyranoside and the less dextrorotatory (**II**) as aniline-N-β-ribopyranoside.

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H. Masamune.

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Studies on Tissue Protease

II. Influence of Metal Salts upon Tryptase Activity of Rabbit Liver

(Directed by Asst. Prof. M. Hayakawa)

By

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Activation by cysteine of catheptase activity is well known, and its activating mechanism has been discussed by many scholars without resulting in definite theory. In this connection influences of various metal salts upon catheptase activity has also been made. Krebs[1] reported that gelatin hydrolysis through catheptase of swine kidney extract has been inhibited by Cu, Ag, Au, Zn, Cd, or by Hg. According to Nakayama[2] who studied catheptic kidney proteolysis, $CuSO_4$ or $HgCl_2$ was most strongly inhibitory. This was also the case when catheptic proteolysis of swine lung was studied by Onishi[3], by whom it was shown that cysteine subdued the inhibitory action of Ag, Cu, or Hg but that no activation by $FeSO_4$ or $FeCl_3$ had occurred.

On the other hand Hayakawa[4] and Ito[5] found that tryptase of human gastric mucosa and rabbit liver, contrary to catheptase, underwent no activation or seemed to be rather inhibited by cysteine. So influence of heavy metal salts upon tryptase is expected to differ from those upon catheptase.

Experimental

Method

The methods are the same as those previously described[4].

The enzyme solution was prepared from liver, from which blood had been removed by means of washing through portal vein with aqua dest.

Reaction mixture: 20 cc. of substrate-buffer (pH 7.8) solution and 3 cc. of enzyme solution were mixed with 3 cc. of solution of various metal salts and then incubated under toluene at 37°C for digestion. Molar concentrations of salt in the digestion mixture are given in the Tables.

For controlling 3 cc. of metal salt solution is substituted with buffer

solution of equal quantity. On the other hand, general control experiments without substrate were carried out with and without metal salt under same condition. Increased activity with respect to each 4 cc. of digestion mixture is formol-titrated with *N*/10-NaOH, and corrected values with general control values are tabulated.

Results with Discussion

$ZnSO_4$ or $Fe_2(SO_4)_3$ appears, in concentration of 1/4,000 *M*, already somewhat to retard the hydrolysis amount after 120 hours' incubation of casein or gelatin, and $HgCl_2$ or $CuSO_4$ appears so in that of 1/2,000 *M*. (Table I). Inhibitory effects of these metal salts are first manifestly observed in concentration of over 1/500 *M*. These inhibitory effects appear to become the more obvious when the times of incubation is the longer.

Inhibitory effect of $AgNO_3$, $NiSO_4$ or of $CoCl_2$ seems less marked

TABLE I

Influence of $ZnSO_4$, $Fe_2(SO_4)_3$, $HgCl_2$ or $CuSO_4$ upon Liver Tryptase Activity of Rabbit at pH 7.8

Increases of acidity in 4 cc. digestion mixture: cc. of 0.1 *N* NaOH.

Salt	Substrate	Time in hrs.	Molar concentration of salts						
			0	1/8,000	1/4,000	1/2,000	1/1,000	1/500	1/1000
$ZnSO_4$	Casein	24	0.15	0.15	0.1	0.1	0.1	0.05	0.05
		72	0.25	0.25	0.2	0.2	0.15	0.1	0.1
		120	0.4	0.35	0.25	0.25	0.2	0.15	0.15
	Gelatin	24	0.15	0.1	0.1	0.1	0.1	0.05	0.05
		72	0.25	0.15	0.15	0.15	0.15	0.1	0.1
		120	0.3	0.2	0.2	0.15	0.15	0.15	0.15
$Fe_2(So_4)_3$	Casein	24	0.1	0.1	0.05	0.05	0.05	0.05	0.05
		72	0.25	0.25	0.25	0.2	0.15	0.1	0.1
		120	0.45	0.45	0.35	0.35	0.3	0.25	0.25
	Gelatin	24	0.1	0.05	0.05	0.05	0.05	0.05	0.05
		72	0.2	0.15	0.15	0.15	0.1	0. 1	0.1
		120	0.3	0.25	0.2	0.2	0.2	0.15	0.15
$HgCl_2$	Casein	24	0.1	0.1	0.1	0.1	0.05	0.05	0.05
		72	0.25	0.25	0.25	0.25	0.1	0.1	0.1
		120	0.3	0.3	0.25	0.2	0.15	0.15	0.15
	Gelatin	24	0.15	0.15	0.1	0.05	0.05	0.05	0.05
		72	0.15	0.15	0.1	0.1	0.05	0.05	0.05
		120	0.2	0.2	0.2	0.2	0.1	0.1	0.1
$CuSO_4$	Casein	24	0.1	0.1	0.1	0.05	0.05	0.05	0.05
		72	0.2	0.2	0.15	0.1	0.1	0.1	0.1
		120	0.3	0.3	0.3	0.2	0.1	0.1	0.1
	Gelatin	24	0.05	0.05	0.05	0.05	0	0	0
		72	0.2	0.2	0.2	0.1	0.1	0.05	0.05
		120	0.25	0.25	0.25	0.15	0.15	0.1	0.1

than above, because retardation of hydrolysis is observed only by 120 hrs.' digestion or in concentration of over 1/500 *M*. (Table II).

$FeSO_4$, $K_4Fe(CN)_6$ and $CdSO_4$ neither inhibit nor activate (Table III).

TABLE II

Influence of $AgNO_3$, $NiSO_4$ or $CoCl_2$ upon Tryptase Activity of Rabbit Liver at pH 7.8

Increases of acidity in 4 cc. digestion mixture; cc. of 0.1 *N* NaOH.

Salt	Substrate	Time in hrs.	Molar concentration of salts						
			0	1/8,000	1/4,000	1/2,000	1/1,000	1/500	1/100
$AgNO_3$	Casein	24	0.1	0.1	0.1	0.1	0.1	0.1	0.1
		72	0.25	0.25	0.2	0.15	0.15	0.15	0.1
		120	0.3	0.25	0.25	0.15	0.15	0.15	0.15
	Gelatin	24	0.05	0.1	0.1	0.05	0.05	0.05	0.05
		72	0.15	0.1	0.1	0.1	0.1	0.1	0.05
		120	0.25	0.25	0.2	0.2	0.2	0.2	0.15
$NiSO_4$	Casein	24	0.1	0.1	—	0.1	—	0.05	0.05
		72	0.2	0.2	—	0.15	—	0.05	0.05
		120	0.3	0.3	—	0.25	—	0.2	0.1
	Gelatin	24	0.05	0.05	—	0.05	—	0.05	0.05
		72	0.15	0.1	—	0.1	—	0.05	0.05
		120	0.2	0.1	—	0.1	—	0.05	0.05
$CoCl_2$	Casein	24	0.1	0.1	—	0.1	—	0.1	0.05
		72	0.2	0.2	—	0.15	—	0.1	0.1
		120	0.3	0.3	—	0.2	—	0.15	0.15
	Gelatin	24	0.05	0.1	—	0.05	—	0.05	0.05
		72	0.15	0.15	—	0.1	—	0.05	0.05
		120	0.25	0.3	—	0.15	—	0.05	0.05

It is, however, noteworthy that $MnSO_4$ seems, in concentration of over 1/2,000 *M*, by 72–120 hours' digestion, to be accelerating for casein or gelatin hydrolysis (Table IV). The effect of $K_4Fe(CN)_6$ is, in concentration of over 1/2,000–1,000 *M*, similarly accelerating (Table IV). But there is some apprehension that one might confuse enzymes, for which activity manganese sulfate is in fact activating, because enzyme solution used contains not only tryptase but also catheptase or ereptase, and manganese sulfate is known to activate dipeptidase activity. Avoiding such confusion an activating effect of manganese sulfate for tryptase is, however, evidenced because the same results are obtained from another experiment (Table V), for which enzyme solution, made by means of acid treatment[5], exclusive of catheptase, ereptase and dipeptidase (Table V–b) is employed.

Next manganese sulfate was examined for its effect in the presence of

TABLE III

Influence of $FeSO_4$, $K_3Fe(CN)_6$ or $CdSO_4$ upon Tryptase Activity of Rabbit Liver at pH 7.8

Increases of acidity in 4 cc. digestion mixture; cc. of 0.1 *N* NaOH.

Salt	Substrate	Time in hrs.	Molar concentration of salts				
			0	1/5,000	1/1,000	1/500	1/100
$FeSO_4$	Casein	24	0.05	0.1	0.1	0.1	0.1
		72	0.15	0.15	0.2	0.2	0.2
		120	0.2	0.2	0.25	0.25	0.2
	Gelatin	24	0.05	0.05	0.05	0.05	0.05
		72	0.1	0.05	0.05	0.05	0.05
		120	0.15	0.1	0.1	0.1	0.2
$K_3Fe(CN)_6$	Casein	24	0.15	0.15	0.15	0.15	0.15
		72	0.25	0.25	0.25	0.25	0.25
		120	0.35	0.35	0.35	0.35	0.35
	Gelatin	24	0.05	0.05	0.05	0.1	0.1
		72	0.15	0.15	0.15	0.15	0.15
		120	0.2	0.15	0.15	0.15	0.15
$CdSO_4$	Casein	24	0.1	0.1	0.1	0.1	0.05
		72	0.15	0.15	0.15	0.1	0.15
		120	0.25	0.2	0.2	0.15	0.2
	Gelatin	24	0.1	0.05	0.05	0.05	0.05
		72	0.15	0.1	0.05	0.05	0.05
		120	0.25	0.2	0.2	0.2	0.2

dialyzed albumin solution, and activating effect observed was duplicated, i.e. the hydrolysis amount became larger than when each of these two activators alone was present (Table VI). But manganese sulfate appears to be somewhat inferior to dialyzed albumin solution in activating effect. From this the suggestion can be made that manganese sulfate may have its own mechanism for activation other than that of dialyzed albumin solution.

Mode of influences of heavy metal salts upon tryptase activity seems, in comparison with those upon catheptase, characteristic in the following respects: Their influences upon tryptase, either activating or inhibiting, appear to be rather delayed, because their influences become more obvious as the time of digestion is increased. Retardations of catheptic hydrolysis are, during the lapse of the time of digestion, more or less restored[2-3]. And the metal salt which is strongly inhibitory against catheptic hydrolysis appears to be not always so against tryptic hydrolysis. Therefore these disimilarities together with the effects of cysteine, suggest that the mechanism of each of these enzyme actions may differ.

On the other hand tryptase in rabbit liver can be definitely said to exist and it manifests itself highly after duplicate activation through dialyzed

albumin solution and manganese sulfate together.

Summary

Influences of metal salts upon liver tryptase activity were investigated with following results:

1. $ZnSO_4$, Fe_2SO_4, $HgCl_2$ or $CuSO_4$ is inhibitory to casein or gelatin hydrolysis through rabbit liver tryptase by 72–120 hours' digestion in concentration of over 1/2000 *M*.

2. The inhibitory effect of $AgNO_3$, $NiSO_4$ or $CoCl_2$ is less than that of the above, and $FeSO_4$, $K_3Fe(CN)_6$ and $CdSO_4$ neither inhibit nor activate.

Table IV

Influence of $MnSO_4$ or $K_4Fe(CN)_6$ upon Tryptase Activity of Rabbit at pH 7.8

Increases of acidity in 4 cc. digestion mixture are expressed in cc. of 0.1 *N* NaOH

Salt	Substrate	Time in hrs.	Molar concentration of salts						
			0	1/8,000	1/4,000	1/2,000	1/1,000	1/500	1/100
$MnSO_4$	Casein	24	0.1	0.1	0.1	0.1	0.15	0.15	0.15
		72	0.2	0.25	0.25	0.3	0.3	0.35	0.35
		120	0.3	0.3	0.35	0.4	0.4	0.45	0.45
	Gelatin	24	0.1	0.15	0.1	0.1	0.1	0.1	0.1
		72	0.15	0.15	0.15	0.2	0.2	0.25	0.25
		120	0.15	0.2	0.2	0.25	0.25	0.3	0.3
$K_4Fe(CN)_6$	Casein	24	0.2	0.2	0.2	0.15	0.15	0.2	0.15
		72	0.25	0.25	0.3	0.35	0.35	0.35	0.35
		120	0.3	0.3	0.3	0.4	0.35	0.4	0.45
	Gelatin	24	0.1	0.1	0.1	0.1	0.1	0.1	0.1
		72	0.2	0.2	0.25	0.25	0.2	0.25	0.25
		120	0.2	0.25	0.25	0.25	0.3	0.3	0.3

Table V

a. Influence of $MnSO_4$ upon Enzyme Solution free from Ereptase and Catheptase Activities

Increased acidity in 4 cc. digestion mixture; cc. of 0.1 *N* NaOH. 5 cc. enzyme solution was used. pH of digestion, 7.8 Substrate, casein

Time in hrs.	Molar concentration of $MnSO_4$				
	0	1/10,000	1/5,000	1/1,000	1/100
24	0.15	0.15	0.15	0.2	0.2
72	0.25	0.3	0.3	0.4	0.4
120	0.35	0.35	0.45	0.5	0.6

b. Proteolytic Activity at pH 7.8 of Tissue Homogenate of Rabbit Liver, exposed to pH 3.5 at 37°C for 40 minutes

Increases of acidity in 4 cc. digestion mixture; cc. of 0.1 *N* NaOH.

Substrate	Time in hrs.	pH of digestion	
		7.8	4.5
Casein	24	0.15	0
	72	0.25	0.05
	120	0.35	0.05
Gelatin	24	0.1	0
	72	0.2	0
	120	0.25	0
Pepton	24	0	0
	72	0	0
	120	0	0
M/20-Diglycin	24	0	0
	72	0	0
	120	0	—

TABLE VI

Activation of Rabbit Liver Tryptase with combined Use of dialyzed Albumin solution and Maganese sulfate solution

10 cc. 2%-casein-buffer solution + 10 cc. dialyzed ov/serum albumin solution or buffer solution + 3 cc. enzyme solution + 3 cc. $MnSO_4$ or buffer solution. Increases of acidity in 4 cc. digestion mixture; cc. of 0.1 *N* NaOH, pH of digestion 7.8.

Time in hrs.	without albumin and $MnSO_4$	with dialyzed serum albumin	with dialyzed ovalbumin	with *M*/100-$MnSO_4$	with dialyzed serum albumin and $MnSO_4$	with dialyzed ovalbumin and $MnSO_4$
24	0.15; 0.1	0.4	0.45	0.35	0.6	0.6 ; 0.5
72	0.25; 0.3	0.65	0.75	0.5	1.05	0.75; 0.75
120	0.35; 0.4	0.9	0.9	0.7	1.35	1.5 ; 1.4

3. $MnSO_4$ or $K_4Fe(CN)_6$ activates casein hydrolysis by 72 hours' digestion in concentration of over 1/1000 *M*. Such effect is recognizable even when catheptase and ereptase are removed by means of acid treatment[6], and manganese sulfate is still activating for tryptase activity which is activated simultaneously in the presence of dialyzed albumin solution. In such a way tryptase is, in its highly active state, undoubtedly recognizable on rabbit liver.

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Studies on Tissue Protease

III. The *in vivo* Activation of Tissue Protease in Rabbit Liver

(Directed by Asst. Prof. M. Hayakawa)

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In the preceding paper it was reported that the activity of tryptase in rabbit tissues is, except in kidney, usually low and in its native state not very manifest[1], and that its remarkable activation in vitro occurs in the presence of dialyzed albumin solution[1,2], manganese sulfate or both[3]. This suggests that tryptase may under some biological conditions be activated in vivo if necessary although the nature or mechanism of activation in vitro is still obscure.

Protease in tissue cells including tryptase should from the physiological point of view play an important role in protein metabolism, because neither the hydrolysis nor the synthesis of the protein could be performed without direct participation of protease system. It would, therefore, be expected that the activation or retardation in vivo of protease system should take place for needs in each tissue where the rate of protein metabolism will increase or decrease.

The author tried, for the first time, to make clear under what condition protease, especially tryptase in rabbit tissues, could be activated in vivo.

Experimental

Methods and Materials

Rabbits fed a normal dict were after fasting twenty four hours given epinephrine through the systemic or portal vein in doses ranging from 0.5 to 4.0 mg. When immediate death did not occur the animals were killed by means of air embolisation.

Details of the enzymological procedure were essentially the same as previously described[1]. Reaction mixture for standard experiment consists with 10 cc. of 2%-substrate-buffer solution (*M*/15-phosphate buffer of pH 4.5 for catheptase), 12 cc. of buffer solution and 2 cc. enzyme solution

(glycerin water (1 :1) 3 : tissue homogenates 1). Substitution of 12 cc. of buffer solution from the standard reaction mixture with 10 cc. of dialyzed albumin solution and 2 cc. of buffer solution are made for albumin activation, with 2 cc. of *M*/20-manganese sulfate solutoin and 10 cc. of buffer solution for manganese activation, with 2 cc. *M*/20-manganese sulfate solution and 10 cc. dialyzed albumin solution for duplicated activation and with 2 cc. *M*/20-cysteine-HCl solution (neutralyzed with *N*/10-NaOH) to pH 4.5 and 10 cc. buffer solution for cysteine activation. Increased acidity with respect to each 4 cc. of reaction mixture is titrated with *N*/10-NaOH after incubation at 37°C under toluene.

Autolysis of liver: Reaction mixture consists of 9 parts of glycerin water (1 :1) and 1 part of liver homogenate. After incubation at 37°C under toluene increased acidity with respect to 10 cc. (*i.e.* 1 g. of liver) is titrated.

The above experiments are always controlled by blank experiments in which substrate or enzyme solution is substituted for buffer solution, and only corrected values are tabulated.

Results

1. *Activation of tryptase or ereptase* (Table I)

By liver proteolysis, the amount of hydrolysis at pH 7.8 of casein is found to be nearly two times higher in those given epinephrine through systemic vein than in those not. Epinephrine appears to produce acceleration of tryptase activity of the liver. In such cases the hydrolysis amount becomes much higher in the presence of dialyzed albumin solution. This indicates that tryptase on liver is augmented by epinephrine. However it is noted that the degree of the in vitro activation appears to be low in early stage of digestion and increasing after relatively long digestion. Usually the degree of activation is almost constant irrespective of digestion time, and this indicates that full activation takes place in the early stage of digestion. The in vivo accelerated liver tryptase, appears to require a long time to attain full activation. The latter is delayed in liver treated with epinephrine than in those not. Such phenomenon may be explained thus: a part of tryptase is activated already in vivo through the same mechanism as that of albumin activatation in vitro.

Coinciding with the results previously obtained[3] manganese sulfate, as well as dialyzed albumin solution, exerts activating effects on tryptase activity of liver which is treated with epinephrine. In the case of duplicated activation with dialyzed albumin and manganese sulfate, the hydrolysis amount becomes much higher.

Tryptase activity is, at any rate, found to be much higher, either in

TABLE I

Influence of Epinephrine Delivery upon Tryptase or Ereptase Activity of Rabbit Liver

Animal (kg.)	Time in hrs.	Hydrolysis* of casein at pH 7.8				Hydrolysis of pepton at pH 7.8
		without albumin or $MnSO_4$	with dialyzed ovalbumin	with 2 cc. $M/20$-$MnSO_4$	with dialyzed ovalbumin & $MnSO_4$	
No. 67 (3.0)	24	0.35	0.4	0.4	0.65	0.8
	72	0.4	0.95	0.6	1.1	1.1
	120	0.6	1.2	1.0	1.5	1.3
No. 57† (3.3)	24	0.2	0.4	0.35	0.5	0.95
	72	0.35	0.7	0.55	0.85	1.2
	120	0.5	1.1	1.05	1.25	1.25
No. 62 (3.2)	24	0.35	0.45	0.5	0.5	0.95
	72	0.7	1.1	1.05	1.6	1.5
	120	0.85	1.5	1.45	1.95	1.6
No. 58‡ (3.1)	24	0.3	0.4	0.45	0.65	0.9
	72	0.45	0.95	0.8	1.15	1.1
	120	0.65	1.25	1.2	1.5	1.2
No. 59 (3.4)	24	0.4	0.45	0.65	0.7	1.05
	72	0.6	0.9	0.9	1.3	1.25
	120	0.75	1.35	1.2	1.65	1.4
No. 69§ (3.3)	24	0.25	0.6	0.4	0.55	0.85
	72	0.4	0.9	0.6	1.0	1.0
	120	0.5	1.0	1.0	1.3	1.05
Normal//	24	0.15	0.3	0.35	0.5	0.7
	72	0.25	0.55	0.5	0.9	1.0
	120	0.35	0.7	0.7	1.1	1.1

* Increases of acidity in 4 cc. digestion mixture; cc. of 0.1 *N*-NaOH. Epinephrine dosage: From above to below, 0.5, 1.0, 2.0, 3.0 and 4.0 mg. respectively.

† Epinephrine delivery is through the portal vein. ‡ Splenectomized animal. § Insulin (200 units) delivery through the systemic vein. // Average of five animals: (Nos. 35, 36, 45, 46 & 47) values presented are fairly consistent with those previously reported.[1]

native state or in fully activated state in those treated with epinephrine than in those not. Whether the nature of albumin activation results from disclosure of the activity after removal of any inhibitory substance or whether it is due to new formation of active enzyme, as was assumed by Matsuo[4], is still obscure. But it is noted that the degree of full activation is found to be nearly the same throughout the experiments, namely always two times increase of the hydrolysis amount is observed irrespective of the epinephrine. The nature of manganese activation may differ from that of dialyzed albumin for the reason previously indicated[3]. At any rate existence on liver of tryptase becomes certain.

Ereptase activity is simultaneously found to increase in animals treated with epinephrine.

2. *Activation of catheptase* (Table II)

In vivo activation of catheptase is, like that of tryptase, shown to occur in the liver of the animals treated with epinephrine. In the case of catheptase proteolysis, hydrolysis of casein is found to be accelerated and that of pepton too, these being further accelerated by cysteine. Catheptic proteinase and catheptic peptonase increased in quantity as a whole.

3. *Activation of liver autolysis* (Table III)

Autolytic proteolysis either at pH 7.8 or 4.5 is also accelerated in those treated with epinephrine. This corresponds with results presented above and is explained as the results of in vivo activation of tryptase, or that of catheptase.

TABLE II

Influence of Epinephrine Delivery upon Catheptase Activity of Rabbit Liver

Animal (body weight, kg.)	Time in hrs.	Hydrolysis* at pH 4.5 of					
		Casein		Gelatin		Pepton	
		without cystein	with cystein	without cystein	with cystein	without cystein	with cystein
No. 67 (3.0)	24	0.45	0.75	0.55	0.85	0.55	0.75
	72	0.7	1.1	0.6	1.05	0.65	0.95
	120	0.9	1.1	0.75	1.05	0.85	1.0
No. 57† (3.3)	24	0.25	0.45	0.25	0.5	0.4	0.6
	72	0.55	0.75	0.35	0.7	0.65	0.85
	120	0.7	0.9	0.6	0.8	0.7	0.9
No. 62 (3.2)	24	0.7	0.95	0.6	0.9	0.6	0.85
	72	1.0	1.55	0.7	1.2	0.75	1.2
	120	1.2	1.8	0.85	1.2	0.95	1.2
No. 58‡ (3.1)	24	0.6	0.95	0.45	0.7	0.4	0.9
	72	0.75	1.05	0.65	0.9	0.75	1.0
	120	1.15	1.5	0.8	1.1	0.9	1.3
No. 59 (3.4)	24	0.6	0.85	0.55	0.8	0.55	0.7
	72	0.75	1.2	0.6	1.0	0.85	1.2
	120	0.85	1.4	0.75	1.15	0.9	1.3
No. 69§ (3.3)	24	0.3	0.5	0.2	0.4	0.4	0.55
	72	0.5	0.65	0.3	0.7	0.5	0.7
	120	0.5	0.8	0.5	0.75	0.6	0.85
Normal//	24	0.25	0.45	0.2	0.5	0.35	0.5
	72	0.35	0.6	0.35	0.6	0.55	0.75
	120	0.45	0.75	0.45	0.7	0.65	0.8

* Increases of acidity in 4 cc. digestion mixture; cc. of 0.1 *N* NaOH. Epinephrine dosage: From above to below, 0.5, 1.0, 2.0, 3.0 and 4.0 mg. respectively.

† Epinephrine delivery is through the portal vein.

‡ Splenectomized animal.

§ Effect of insulin (200 units, intravenously) is observed.

// Average of three animals (Nos. 45, 46 & 47).

TABLE III

Influence of Epinephrine Delivery upon Autolytic Proteolysis of Rabbit Liver

Increases of acidity with respect to 1 g. Liver; cc. of 0.1 *N* NaOH.

pH of autolysis	Time in hrs.	Autolytic proteolysis of the liver of animal						
		No. 67	† No. 57	No. 62	* No. 58	No. 59	‡ No. 69	(Normal) Nos. 45, 64, 46 & 47
4.5	24	1.8	1.65	2.3	1.8	1.75	1.5	1.5 ; 1.4 ; 1.5 ; 1.55
	72	2.6	2.3	2.95	2.7	2.6	2.25	2.1 ; 2.15; 2.2 ; 2.1
	120	2.75	2.8	3.45	2.95	2.9	2.75	2.5 ; 2.5 ; 2.55; 2.5
7.8	24	0.55	0.5	0.9	0.85	0.8	0.5	0.4 ; 0.3 ; 0.35; 0.4
	72	0.7	0.75	1.4	1.1	1.1	0.75	0.55; 0.55; 0.5 ; 0.55
	120	1.2	1.2	1.55	1.3	1.3	1.0	0.9 ; 0.85; 0.85; 0.8

Epinephrine dosage: From left to right, 0.5, 1.0, 2.0, 3.0 and 4.0 mg. respectively.

* Splenectomized animal.

† Epinephrine delivery is through the portal vein.

‡ Effect of insulin (200 units, intravenously) is observed.

4. *Effect of insulin etc.*

Insulin in the dosis employed produces slightly augmentation of tryptase, but ereptase or catheptase activity remain unaffected (Tables I, II & III, animal No. 69). This may be interpreted as the results of epinephrine release following hypoglycemic state.

Direct delivery of epinephrine to liver through the portal vein has only a slight effect on the liver protease system (Tables I, II & III, animal No. 57). This may due to destruction of epinephrine, as indicated by Sherlock[5] who showed that direct delivery of epinephrine to the liver caused only slight change in blood glucose and did not affect the liver glycogen content.

5. *Histological examination*

Histological examination of the liver delivered with epinephrine reveals only slight congestion of blood, this being in no relation to its protease activity. Therefore, it is not likely that enzyme augmentation observed above results simply from immigration of blood cells containing proteolytic enzyme from periphery to liver. Accordingly augmentation of protease including that of tryptase should be considered to occur in the liver cell itself upon epinephrine delivery, and this gives further foundation for the existence of tissue tryptase in liver cell and suggests the possibility of the formation of new enzyme molecules within tissue cells under certain physiological conditions.

6. *Proteolysis of gastric mucosa or kidney* (Tables IV & V)

TABLE IV

Influence of Epinephrine Delivery upon Tryptase or Ereptase Acidity of Rabbit Kidney

Animal No.	Time in hrs.	Hydrolysis* at pH 7.8					
		without activation			with albumin activation†		
		Casein	Gelatin	Pepton	Casein	Gelatin	Pepton
67	24	0.4	0.35	1.15	1.0	0.6	1.15
	72	1.05	0.65	1.3	2.15	1.45	1.25
	120	1.3	1.05	1.3	2.5	2.15	1.35
57‡	24	0.3	0.35	1.1	0.65	0.7	1.15
	72	1.0	0.65	1.2	2.05	1.6	1.2
	120	1.25	1.1	1.3	2.55	2.35	1.3
62	24	0.4	0.3	1.25	0.85	0.65	1.15
	72	1.05	0.7	1.1	2.15	1.65	1.2
	120	1.15	1.25	1.3	2.6	2.35	1.35
58§	24	0.35	0.35	1.1	0.8	0.8	—
	72	1.1	0.7	1.25	2.0	1.4	—
	120	1.25	1.1	1.25	2.25	2.25	—
59	24	0.4	0.35	1.1	0.9	0.55	—
	72	1.0	0.65	1.3	2.35	1.7	—
	120	1.25	1.1	1.3	2.8	2.2	—
69//	24	0.35	0.35	1.05	0.65	0.75	1.1
	72	1,0	0.7	1.15	2.1	1.65	1.2
	120	1.3	1.1	1.25	2.65	2.25	1.3
Normal¶	24	0.35	0.35	1.1	0.65	0.75	1.15
	72	1.05	0.7	1.25	2.1	1.65	1.2
	120	1.25	1.1	1.25	2.65	2.25	1.35

* Increases of acidity in 4 cc. digestion mixture; cc. of 0.1 *N* NaOH. Epinephrine dosage: From above to below, 0.5, 1.0, 2.0, 3.0 and 4.0 mg. respectively.

† Dialyzed serum albumin solution is used.

‡ Epinephrine delivery is through the portal vein.

§ Splenectomized animal.

// Effect of insulin is observed.

¶ Average of three animals -Nos. 45, 46 and 47). The values are fairly consistent with those obtained previously.[1)]

Epinephrine delivery, through either systemic or portal vein, exerts no influence upon proteolysis of gastric mucosa or kidney. Therefore enzyme augmentation phenomenon seen above appears to be specific to liver. Influence of insulin delivery is not seen.

DISCUSSION

Toenniessen[6)] stated early that sympathic nerves accelerated protein catabolism in the liver and in this connection the above augmentation phenomenon caused by epinephrine in the liver protease system may be explained as corresponding with an increase of the rate of protein metabol-

TABLE V

Influence of Epinephrine Delivery upon Tryptase or Ereptase Activity of Rabbit Gastric Mucosa

Animal No.	Time in hrs.	Hydrolysis* at pH 7.8					
		without activation			with albumin activation†		
		Casein	Gelatin	Pepton	Casein	Gelatin	Pepton
67	24	0.2	0.1	0.45	0.35	0.15	0.4
	72	0.35	0.5	0.55	1.0	0.75	0.55
	120	0.45	0.5	0.6	1.0	0.85	0.55
57‡	24	0.1	0.1	0.35	0.35	0.25	0.5
	72	0.35	0.35	0.5	0.7	0.6	0.5
	120	0.4	0.4	0.5	0.9	0.95	0.55
62	24	0.2	0.15	0.35	0.45	0.2	—
	72	0.35	0.4	0.55	0.75	0.8	—
	120	0.45	0.45	0.55	1.0	1.0	—
58§	24	0.1	0.1	0.4	0.3	0.35	—
	72	0.35	0.35	0.6	0.85	0.75	—
	120	0.4	0.5	0.55	0.95	1.05	—
59	24	0.15	0.1	0.4	0.35	0.35	—
	72	0.3	0.35	0.55	0.75	0.7	—
	120	0.45	0.5	0.6	1.05	1.15	—
69//	24	0.15	0.1	0.35	0.25	0.25	0.4
	72	0.35	0.35	0.5	0.7	0.8	0.55
	120	0.4	0.4	0.55	0.8	0.8	0.6
Normal¶	24	0.15	0.1	0.35	0.35	0.25	0.35
	72	0.35	0.4	0.55	0.85	0.8	0.5
	120	0.4	0.5	0.55	1.0	1.0	0.55

* Increases of acidity in 4 cc. digestion mixture; cc. of 0.1 *N* NaOH. Epinephrine dosage: From above to below, 0.5, 1.0, 2.0, 3.0 and 4.0 mg. respectively.

† Dialyzed serum albumin solution is used.

‡ Epinephrine delivery is through the portal vein.

§ Splenectomized animal.

// Effect of insulin is observed.

¶ Average of three animals (Nos. 45, 46 and 47). The values are fairly consistent with those obtained previously.[1]

ism in the liver, because protein metabolism, either catabolism or anabolism should be controlled under activities of proteolytic enzyme system. The latter should, therefore, be a direct indication of the former. From such a view point it is easy to conceive that such increase of the rate of protein metabolism might correlate with gluconeogenesis which follows glycogenolysis after consumption of glycogen deposit in liver, because epinephrine is known to accelerate enzymatic conversion of glycogen to glucose causing hyperglycemia.

Since pH value of tissue cells which is assumed usually to be nearly 7.3–7.4 seems of more advantage for tryptic hydrolysis, augmentation of

tryptase may be interpreted by an increase of the rate of protein catabolism in the liver. Therefore the first step of reaction for gluconeogenesis should be suggested to be hydrolysis of protein to amino acid in the liver. If gluconeogenesis is progressing further, protein deposit in liver may gradually fall, and protein resynthesis, together with hydrolysis, should be effected and augmentation of catheptase may be interpreted by an increase of the rate of protein anabolism in the liver. Such explanation is consistent with Schoenheimer's finding[7] that intracellular proteins including liver protein continually undergo breakdown and synthesis. And it is very interesting that Sutherland and Cori[8] demonstrated that epinephrine caused augmentation of phosphorylase in the liver either in vitro or in vivo and that phosphorylase reaction was the rate limiting step of glycogenolysis.

Summary

1. Liver tryptase activity (casein hydrolysis at pH 7.8) of rabbit treated with epinephrine through the systemic vein increases markedly, its hydrolysis amount becoming two times higher than that of those not treated. Such increased activity can be further activated in vitro in the presence of dialyzed albumin, manganese sulfate or the both.

2. Activity of ereptase (pepton hydrolysis at pH 7.8) and catheptase (casein, gelatin or pepton hydrolysis at pH 4.5) in animals so treated is higher than in those not treated. Increased activity of catheptase is further activated in vitro by cysteine.

3. Autolytic proteolysis eithr at pH 4.5 or 7.8 also increases markedly after epinephrine treatment.

4. Direct delivery through the portal vein to the liver exerts only a slight effect on the liver protease system. Insulin delivery produces slight augmentation of tryptase, but ereptase or catheptase remains unaffected. Spleen extirpation does not influence on epinephrine effect.

5. Histological examination of the liver delivered with epinephrine reveals only slight congestion of blood, this being in no relation to its protease activity.

6. Epinephrine or insulin delivery through either systemic or portal vein exerts no influence upon proteolysis of gastric mucosa or kidney.

7. Above augmentation phenomenon caused by epinephrine in liver protease system, it is suggested, relates to gluconeogenesis in liver.

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Studies on Tissue Protease

IV. The *in vivo* Activation of Protease System and Gluconeogenesis In Liver

(Directed by Asst. Prof. M. Hayakawa)

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In the preceding paper[1] it was demonstrated that epinephrine produced remarkable augmentation of proteolytic enzyme system on liver, and it was suggested thereby that such phenomenon might relate to gluconeogenesis which should be carried into effect immediately after consumption of glycogen deposit in liver. However, the relation to gluconeogenesis of adrenal cortex has been suggested by Long[2] and the protein catabolic effect of adrenal cortical substances is now rather common knowledge. On the other hand epinephrine is known as an effective activator for the pituitary-adrenal system. Regarding these matters further studies were made on in vivo activation of the liver protease system.

Experimental

The method was the same as the one employed in the preceding paper[2].

Results

Coinciding with preceding observations, epinephrine delivery through the systemic vein results in acceleration of heterolytic and autolytic proteolysis of liver and causes augmentation of tryptase as well as that of catheptic protease (Tables I, II & III, Animal, No. 73). In this case the animal was killed four hours after epinephrine delivery, so its effect is shown to be able to continue at least four hours. Blood cell counts of the animal before and after the delivery are shown in Table IV. Eosinophil cell and lymphocyte counts fall apparently four hours after the delivery, i.e., response to the latter of its pituitary-adrenal system appears to be well preserved.

To determine whether or not epinephrine exerts its effect on liver protease system via such endocrine system, further experiments were carried

TABLE I

Epinephrine Effect on Tryptic Proteolysis of Rabbit Liver at pH 7.8

(Increases of acidity in 4 cc. digestion mixture (cc. of 0.1 *N* NaOH))

Substrate*	Time in hrs.	Animal (Source of enzyme solution)							
		No. 73†	adrenalectomized			glucose administered			Normal control‡
			No. 61	No. 72	No. 74	No. 68	No. 70	No. 71	
Casein (a)	24	0.25	0.35	0.35	0.35	0.15	0.15	0.15	0.15
	72	0.55	0.6	0.7	0.7	0.35	0.35	0.3	0.25
	120	0.75	0.8	0.85	0.8	0.35	0.4	—	0.35
Casein (b)	24	0.3	0.35	0.4	0.4	0.3	0.3	0.3	0.3
	72	1.2	1.25	1.2	1.1	0.6	0.75	0.65	0.55
	120	1.45	1.55	1.5	1.5	0.8	0.8	—	0.7
Casein (c)	24	0.45	0.55	0.5	0.45	0.4	0.35	0.3	0.35
	72	0.9	0.95	0.9	0.8	0.55	0.65	0.65	0.5
	120	1.2	1.3	1.35	—	0.75	0.8	—	0.7
Casein (d)	24	0.7	0.75	0.7	0.1	0.55	0.6	0.6	0.5
	72	1.4	1.5	1.55	1.4	0.95	0.95	1.05	0.9
	120	1.75	1.85	1.85	—	1.05	1.1	—	1.1
Gelatin (a)	24	0.2	0.2	0.2	0.3	0.1	0.1	0.1	0.1
	72	0.25	0.3	0.25	0.3	0.2	0.2	0.15	0.2
	120	0.35	0.35	0.35	0.35	0.3	0.25	0.2	0.3
Gelatin (b)	24	0.3	0.3	0.35	0.3	0.3	0.25	0.15	0.25
	72	0.6	0.65	0.65	0.65	0.4	0.4	0.35	0.4
	120	0.75	0.8	0.85	0.85	0.5	0.5	0.55	0.6
Pepton (a)	24	0.7	0.9	0.9	0.95	0.75	0.65	0.6	0.7
	72	1.4	1.6	1.7	1.7	0.9	0.8	0.8	1.0
	120	1.5	1.6	1.7	1.7	0.95	0.95	0.95	1.1

Epinephrine doses: 2.0 mg. intravenously.

Animal: No. 73, 3.0 kg.; No. 61, 3.2 kg.; No. 72, 3.2 kg.; No. 74, 3.4 kg.; No. 68, 2.3 kg.; No. 70, 3.0 kg.; No. 71, 3.3 kg.

* (a) without albumin or manganese activation; (b) with dialyzed serum albumin solution; (c) with manganese activation (*M*/10-$MnSO_4$ solution); (d) with combined activation with dialyzed serum albumin and manganese sulfate solutions.

† Killed 4 hours after the epinephrine delivery.

‡ Average of five animals (Nos. 35, 36, 45, 46 and 47).

out on animals adrenalectomized. On such animals, too, epinephrine casuses augmentation of liver protease system, and such effect is independent of pituitary-adrenal cortical system (Tables I, II & III, Animals: Nos. 61, 72 & 74). Epinephrine itself exerts, however, no influence upon liver proteolysis in vitro when it is added directly with liver homogenates (Table V). Therefore its effect in vivo is indirect.

If such augmentation could be related directly to gluconeogenesis in

TABLE II

Epinephrine Effect on Catheptic Proteolysis of Rabbit Liver at pH 4.5

(Increases of acidity in 4 cc. digestion mixture; cc. of 0.1 *N* NaOH)

Substrate*	Time in hrs.	Animal (Source of enzyme solution)							
		No. 73†	adrenalectomized			administered glucose			Normal control‡
			No. 61	No. 72	No. 74	No. 68	No. 70	No. 71	
Casein (a)	24	0.5	0.6	0.6	0.65	0.35	0.3	0.35	0.25
	72	1.05	1.05	1.05	0.95	0.4	0.4	0.45	0.35
	120	1.2	1.2	1.2	1.25	0.55	0.5	0.55	0.45
Casein (b)	24	0.9	0.95	0.9	—	0.55	0.5	—	0.45
	72	1.55	1.3	—	—	0.7	—	—	0.6
	120	1.8	1.7	—	—	0.8	—	—	0.75
Gelatin (a)	24	0.65	0.7	0.75	0.65	0.35	0.3	0.25	0.2
	72	0.75	0.8	0.85	0.8	0.45	0.35	0.3	0.35
	120	0.9	0.9	0.9	0.9	0.6	0.55	0.6	0.45
Gelatin (b)	24	0.9	0.9	—	—	0.55	—	—	0.5
	72	1.2	1.25	—	—	0.85	—	—	0.7
	120	1.25	1.25	—	—	0.85	—	—	0.7
Pepton (a)	24	0.55	0.6	0.5	0.5	0.4	0.3	0.35	0.35
	72	0.7	0.7	0.7	0.7	0.45	0.4	0.5	0.55
	120	0.9	0.95	0.9	0.9	0.55	0.6	0.6	0.65
Pepton (b)	24	0.75	0.85	—	—	0.55	—	—	0.5
	72	1.0	1.2	—	—	0.7	—	—	0.75
	120	1.1	1.3	—	—	0.8	—	—	0.8

Epinephrine dose: 2.0 mg. intravenously.

Animal, body weight (kg.): No. 73, 3.0; No. 61, 3.2; No. 72, 3.2; No. 74, 3.4; No. 68, 2.3; No. 70, 3.0; No. 71, 3.3.

* (a) without cystein, (b) with cystein activation.

† Killed 4 hours after the epinephrine delivery.

‡ Average of three animals (Nos. 45, 46 and 47).

liver it would be expected that the effect of epinephrine would be abolished under some conditions. Hyperglycemic state, for example, may account for such a condition, because there should be no need for gluconeogenesis and so none for protease augmentation. Therefore the effect of epinephrine was examined on three animals injected with 20 cc. of 10%-glucose solution and kept beforehand in a hyperglycemic state (urine sugar). After epinephrine delivery blood sugar rose further of the three animals and fell in one animal (Table VI), and activity of liver protease system was, as was expected, by no means accelerated (Tables I, II & III, Animals, Nos. 68, 70 & 71). Effect on liver protease system of epinephrine was thus abolished by prior administration of glucose solution.

TABLE III

Epinephrine Effect on Catheptic Proteolysis of Rabbit Liver

(Increases of acidity in cc. of 0.1 *N* NaOH for 1 g. liver.)

pH of Autolysis	Time in hrs.	Autolytic proteolysis of the liver of animal							
		No. 73*	adrenalectomized			administered with glucose			Normal control†
			No. 61	No. 72	No. 74	No. 68	No. 70	No. 71	
4.5	24	1.9	2.2	2.2	2.15	1.5	1.4	1.55	1.5
	72	2.7	2.8	2.85	2.95	2.15	2.1	2.1	2.1
	120	3.0	3.25	3.3	3.45	2.45	2.4	2.5	2.5
7.8	24	0.9	1.05	0.95	0.95	0.4	0.45	0.4	0.4
	72	1.1	1.25	1.25	1.25	0.7	0.6	0.6	0.55
	120	1.35	1.5	1.45	1.65	0.9	0.9	0.85	0.85

Epinephrine dose: 2.0 mg. intravenously.

* Killed 4 hours after the epinephrine delivery.

† Average of three animals (Nos. 45, 46 and 47).

TABLE IV

Blood Picture of Animal No. 73 before and after the Epinephrine Delivery*

	Before	After
Eosinophil† cell count	250	75
Leucocyte count	15,000	22,000
Lymphocyte count (%)	5,150 (52) %	3,630 (17.5) %
Neutrophil cell count (%)	4,050 (40.5) %	17,380 (79.0) %

* Animal No. 73, 3.0 kg. delivered with 2.0 mg. epinephrine through the system vein and killed four hours after the delivery.

† According to Dunger's method.

Prior administration of 10 cc. of 10%-alanin solution which was thought to be a effective precursor of glucose was also shown to prevent similarly augmentation of tryptase or retard that of catheptase, and epinephrine exerts no remarkable effect on tryptic proteolysis of the liver of animal treated with it a short time after a meal (Table VII).

Therefore, the above assumption that enzyme augmentation may relate to gluconeogenesis appears to be founded in these facts observed.

Epinephrine is, on the other hand, known also to accelerate glycogenolysis of muscle, therefore, its effect on muscle proteolysis was examined.

TABLE V

Influence of Epinephrine upon Liver Proteolysis* in vitro

(Increases of Acidity in 4 cc. digestion mixture; cc. of 0.1 *N* NaOH)

Substrate	Time in hrs.	0.1%-epinephrine, cc.			
		0	0.5	1.0	2.0
2% Casein	24	0.15	0.15	0.15	0.15
	72	0.25	0.3	0.25	0.25
	120	0.35	0.35	0.3	0.35
2% Pepton	24	0.6	0.6	0.65	0.6
	72	0.75	0.7	0.75	0.7
	120	0.9	0.95	0.85	0.9

* Normal liver (animal No. 63, 3.2 kg.).

20 cc. substrate-buffer (pH 7.8) solution+2 cc. enzyme solution (liver homogenate 1 : glycerin water 3)+0—2.0 cc. epinephrine solution+2.0—0 cc. buffer solution

Epinephrine treatment for enzyme solution :

2 cc. original enzyme solution was added with 0—2.0 cc. 0.1%-epinephrine solution and 2.0—0 cc. buffer solution and incubated at 37°C for forty minutes (final volume being made 4 cc. respectively).

TABLE VI

Change of Blood Sugar (mg/dl) after Glucose Administration and Epinephrine Delivery

Animal No.	Before	After glucose	After epinephrine
68	114	460	480
70	121	509	380
71	129	509	543

Muscle tryptase is, in native state, low in activity as was so when previously observed[8]. In the case of epinephrine or insulin delivery no change in tryptase or ereptase activity was observed (Table VIII).

Discussion

Although catabolic effect of adrenal cortical substance is well established, relationship between this and gluconeogenesis or details of biological mechanism of the latter has not yet been elucidated.

From the results presented above epinephrine is evidenced to cause, independent of the adrenal cortex, augmentation of the liver protease system and this should be interpreted as indicating increase in the rate of protein metabolism which should directly relate to gluconeogenesis ad-

TABLE VII

Influence of Prior Administration of Alanin etc. upon Epinephrine Effect on Rabbit Liver Proteolysis

(Increases of acidity in 4 cc. digestion mixture (cc. of 0.1 *N* NaOH)).

Animal No.	Time in hrs.	pH 7.8				pH 4.5		
		Casein (a)*	Casein (b)†	Gelatin	Pepton	Casein	Gelatin	Pepton
86‡	24	0.15	—	0.1	0.45	—	—	—
	72	0.2	—	0.15	0.95	—	—	—
	120	0.3	—	0.15	1.0	—	—	—
106§	24	0.1	0.2	0.1	0.45	0.3	0.35	0.25
	72	0.2	0.3	0.15	0.75	0.45	0.6	0.5
	120	0.2	0.5	0.15	0.75	0.65	0.7	0.55
Normal//	24	0.15	0.3	0.1	0.7	0.25	0.2	0.35
	72	0.25	0.55	0.2	1.0	0.35	0.35	0.55
	120	0.35	0.7	0.3	1.1	0.45	0.45	0.65

* Without albumin.
† With dialyzed serum albumin solution.
‡ Epinephrine delivery short time after meal.
§ Prior administration of 1 g. alanin.
// Cf. Tables I and II.

TABLE VIII

Influence of Epinephrine upon Tryptic Proteolysis of Striped Muscle of Rabbit

(Increases of acidity in 4 cc. digestion mixture (cc. of 0.1 *N* NaOH)).

Animal No. (body weight, kg.)	Time in hrs.	Hydrolysis at pH 7.8 of			Remarks
		Casein	Gelatin	Pepton	
64 (3.2)	24	0.2	0.05	0.25	normal control
	72	0.3	0.1	0.45	
	120	0.35	0.25	0.6	
67 (3.0)	24	0.1	0.05	0.2	0.5 mg. epinephrine intravenously
	72	0.25	0.1	0.45	
	120	0.4	0.2	0.6	
68 (2.3)	24	0.1	0.05	0.3	glucose (10 g.) administration prior to epinephrin delivery (2 mg.)
	72	0.25	0.15	0.45	
	120	0.35	0.2	0.55	
69 (3.3)	24	0.15	0.05	0.3	Insulin delivery (200 units)
	72	0.2	0.15	0.55	
	120	0.3	0.15	0.6	

vancing therein. From such point of view it is concluded that gluconeogenesis in the liver does not require the presence of adrenal cortex. Selye[4]

is of the opinion that the corteicoid aids rather the utilization of protein catablites for gluconeogenesis or protein re-synthesis. And it was concluded by Ingle[5] that the negative nitrogen balance after traumatic injury is not caused specially by the increase in secretion of the cortical hormones.

Referring to catabolic effect of adrenal cortex, Bondy[6] showed that it did not require the presence of the liver. That epinephrine causes augmentation of the liver protease system independent of adrenal cortex is, therefore, compatible with Bondy's results. According to Selye[4] the breakdown of body protein, except those of lymphocytes, takes place independent of the adrenal cortex. White and Doughtery[7] showed on fasting mice that a decrease in nitrogen content was seen in livers of adrenalectomized animals as well as in those not operated on. These observations show the possibility that protein metabolism of the liver can progress regardless of adrenal cortex and that it is controlled by a mechanism other than that of metabolism of the other body protein. From the results obtained, epinephrine, namely adrenal medulla or sympathic nerve, is concluded to be a factor which controls the metabolism of liver protein.

But it is very interesting that glucose or alanin is antagonistic to the effect of epinephrine, as was so in the experiments conducted by Engel[8] on the effect of adrenal cortical extract. Bondy[6] showed such effect of glucose to be carried out in the periphery to the liver, but detail of mechanism of antagonistic effect against epinephrine of glucose or alanin is open to question. These substances are, at least, said to spare the liver protein in occasion of " emergency reaction ".

Engle[8] considered the effect of adrenal cortex on protein metabolism was secondary to the changes in the carbohydrate/fat metabolism. From the above the effect of epinephrine should be indirect upon the protease system itself, and the augmentation of liver protease system, namely, the increase in the rate of protein metabolism, should also be considered to be brought into effect secondary to glycogenolysis and corresponding with gluconeogenesis directly. It is suggested that gluconeogenesis occurs first with consumption with liver protein. The protein catabolites which could come from other tissue proteins under adrenal cortical influence may serve for the re-synthesis of liver protein or directly for gluconeogenesis. The role of adrenal cortex for gluconeogenesis may be explained as supplying amino acid to the liver and or sparing liver protein.

Summary

1. Epinephrine delivery in vivo causes augementation of the liver protease system on animals fasted for twenty four hours. But no change in protease activities is seen in vitro when epinephrine is directly added to

liver homogenates.

2. Such phenomenon is observed in the absence of adrenal cortex but not observable on animals treated with epinephrine a short time after a meal.

3. Prior administration of glucose or alanin prevents epinephrine effect, i.e., no augmentation of protease system is seen.

4. Tryptic muscle proteolysis remains unaffected after epinephrine treatment.

5. The biological mechanism of gluconeogenesis is briefly discussed with enzyme augmentation phenomenon.

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EXPERIMENTAL PATHOLOGY OF DIABETES MELLITUS

(REPORT II)

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PREFACE

In 1949, I published for the first time my Zinc Theory of Diabetes Mellitus by Okamoto, based on the findings and observations we had obtained through our past studies and experiments on diabetes mellitus. Since then, we have been engaged in the research in the same field without intermission. The results we had achieved in our experiments up to the end of 1950 were made public in Japanese in my monogram entitled Experimental Pathology of Diabetes Mellitus, Nihon Isho Shuppan, Tōkyō and Kyōto.

Ever since, I continued my study, with the cooperation of the staff of my laboratory, and it is my pleasure that I can state I have been further corroborated in the theory I have proposed in 1949.

Hereunder, I am going to report on the three key problems of the studies I have been engaged in since then, namely, the research on the diabetogenic substances, the research on the diabeto-prophylactic substances and the research on the off-springs of diabetic animals. I have numbered this report No. II, because I wish to have my previous monogram cited above referred to as my first report. We are of course keeping up our research with unflagging zeal, and hope to publish our Reports III, IV and infinitum in the future.

I herewith wish to express my heartiest thanks to my co-workers Messrs. T. Fujiwara, K. Fukutome, T. Hayakawa, T. Ibaraki, H. Kōso, K. Sukenari, T. Taii, N. Takenaka and Y. Tani for their painstaking cooperation in my experimental works, to Prof. S. Mori of Kyōto University, Prof. R. Shōji (President of Kōbe Medical College), Prof. M. Tomita of Kōbe University and Prof. T. Shimizu of Okayama University for their unfailing and kind suggestions and encouragements.

I must mention with gratitude that this study was made possible by allocation of Scientific Research Fund by the Education Ministry and by the Asahi Scientific Scholarship Fund. Appreciation is also due to Mr. T. Matuo of Kōbe Women's College of Pharmacy for having prepared the synthesised reagents for me.

Sendai
March, 1955

Kōzō Okamoto

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I. Experimental Studies on Production and Progress of Diabetes Mellitus by Zinc Reagents*

Okamoto[1] has discovered that a large quantity of zinc is contained in the Langerhans islets of the pancreas by histochemical demonstration, using diphenylthiocarbazide, of zinc in tissue, a method he has perfected himself (1942). The zinc content of pancreas is somewhat variable according to the kind, the age and the bodily conditions of the animal,[2] but it must be noted that the zinc is present in a form ready to react and combine with zinc reagents, that is, with zinc-affine substances, and not in the form of compounds in which zinc is closely combined with organic substances.

Reasoning on this finding with the fact that alloxan, that has the property to injure the β-cells of the Langerhans islets selectively and thus is a strong diabetogenic reagent,[3,4] is a chemical ready to combine with zinc,[5] the following idea has been deduced (1949) :[6]

When a zinc-affine substance is brought into blood it will certainly combine with the zinc content in the Langerhans islets. Now, when the substance is cytotoxic, the cells here will be injured, resulting in weakened insulin production and inducing diabetes. If, however, this substance is not toxic to cells, it will not injure the cells, and as it comes into combination with the zinc in the Langerhans islets, it will prevent the combination of any zinc-affffine or diabetogenic substance subsequently brought in and thus prevent the occurrence of diabetes.

For this idea to be valid, it is of course prerequisite that the zinc content of the Langerhans islets should be in a form ready to react and combine with zinc-affine substances, but it is also required that the zinc-affine reagent brought into the circulating blood should meet various necessary conditions, such as, that it is soluble in blood and humor, that its reaction with zinc is very sensitively and speedily effected, that its reaction is completed in neutral or slightly alkaline environment at a temperature of 37°C or so, that it does not readily combine with other substances than zinc in the body and that it does not decompose in or is not excreted too readily out of the body.

Acting upon such a conception, Okamoto, with the help of Kadota, made experimental studies with zinc reagents and discovered oxine and dithizone to be diabetogenic and reported it in 1949[6] (Kadota[7] has made a more detailed report in 1950), and also under the suggestion of Okamoto, Ibaraki and Shibata in his laboratory made clear that anthranilic and quinaldinic acids act prophylactic against diabetization by zinc reagents.[6] Hereupon, Okamoto was convinced of the rightness of the above hypothesis of his and concluding that zinc is contained in the Langerhans islets and this is closely related with the

* By Kōzō OKAMOTO, Tadayoshi FUJIWARA, Kenzō SUKENARI, Yutaka TANI and Kin-ichirō FUKUTOME.

production and prevention of diabetes mellitus, has published it as the "Zinc Theory of Diabetes Mellitus by Okamoto" in 1949.[6]

Since then, he has been continuing experimenting with zinc affine substances under the cooperation of his coworkers, and have ascertained the diabetogenetic effect of nicotine (1951),[8,9,10] and 13 varieties of quinoline compounds (1951, 1952),[11,12,13,14] and identified more than 20 chemicals as prophylactic against alloxan diabetes (1952).[6,8,12] These findings were made public in summaries at the meetings of the Japanese Pathological and the Japanese Biochemical Associations. As the histological studies have come to a conclusion, for the time being, we wish to make a general report summarizing our findings, in particular on how the diabetogenic reagents act in producing the illness and how it progresses when diabetes is induced in animals. On the diabetesprophylactic substances, we will speak in the next chapter.

Experimental

Materials and Method

Animals and their feeding: Chiefly, adult rabbits of 1,900~3,500g. in bodyweight were used. Some adult mice of 15~25 g. were also made use of. The experimental rabbits were kept in large wooder boxes and fed for more than a week at our laboratory with a sufficient diet of bean-cake refuse, vegetables and grass. The feed was taken off on the eve of the administration of the reagents. The reagents were always administered before noon, and thereafter, beside the above diet, oats, sweet-potatoes and carrots were freely fed and water and a small quantity of fish meal were also supplied.

The mice were mainly used for the determination of lethal doses and the observations concerned with the combination of zinc in Langerhans islets by vital staining. They were kept in smaller boxes and given sufficient rice, barley, fish meal, vegetables and water to feed upon.

Tested reagents: As the experiments for diabetization were conducted under the hypothesis stated in the introduction, the tested reagents were mainly those ready to react and combine with zinc. These included, beside the chemicals used as zinc reagents in analytic chemistry,[15,16,17,18] also some chemicals of which the zinc-affinity we have ascertained in vitro ourselves. Of course, these had to include chemicals very sensitive in their reaction with zinc and some quite otherwise. We have also excluded such reagents that could not be used in injection in animals, owing to the inappropriateness of the solvents. We have also tried some chemicals that are not zinc-affine but similar in chemical structure to diabetogenic reagents or zinc reagents, and some chemicals little affine with zinc but known as excellent reagents of metals nearly related to zinc or thought to be contained in the Langerhans islets beside zinc, such as Cu, Cd, Co and Ni.[15,16] In total, the number of tested reagents amounted to 87. These were used in fresh solutions dissolved in distilled water, dilute hydrochloric acid or dilute alkali, under room temperature or upon applying heat.

For observing the progress of diabetes in rabbits, we used also known diabetogenic reagent, such as alloxan (alloxan monohydrate marketed by East-

man Kodak Co. The new brand labelled "Distillation Products" have the efficacy of diabetization in doses of 100~120 mg./kg.), oxine (manufactures of Takeda or Hayashi) and dithizone (made by Merck or Takeda), alongside the reagents discovered as diabetogenic in our experiments.

Experimental Method: In the studies reported herewith, we investigated the efficacy of sundry chemicals in inducing diabetes in rabbits, the progress of illness thus induced and also how the reagents come to be fixed in the Langerhans islets, that is how the islets are vitally stained by colored reagents. The methods used were as follows:

1) In the diabetization tests, we first ascertained the approximate lethal doses of the tested reagent by intravenal injection into the tail of mice. Then we injected rabbits with nearly the same doses through the auricular vein, and tested the rabbits for their symptoms, blood sugar and urinal sugar. When a rabbit died during the experiment or was sacrificed after the lapse of a fixed time, its entrails, especially its pancreas, were examined histologically. Then the doses were increased or decreased and the degree or the absence of produced pancreatic diabetes and the injury to the Langerhans islets estimated. Diabetes was deemed produced when blood sugar of more than 200 mg./dl. and conspicuous glycosuria were verified continuously for several days and frank injury was discovered in Langerhans islets.

2) In observing the progress of the illness in rabbits, rabbits made diabetic by administration of a newly discovered efficacious diabetogenic reagent or intravenal injection of 200 mg./kg. of alloxan,[4] 50~60 mg./kg. of oxine[6,7] or 100 mg./kg. of dithizone[6,7] were selected and subjected to observation without insulin administration (insulin therapy was applied only when aceton compounds began to be excreted in urine). Sugar and aceton compounds in their urine were tested everyday and their bodyweight and blood sugar at appropriate intervals. When without markedly depressed appetite a state of less than 120 mg./dl. of sugar in blood and negative sugar in urine persisted, the rabbit was judged as cured. When a rabbit died during the observation, it was dissected and it entrails examined, the cause of death and the state of the Langerhans islets being investigated in particular.

3) Observation of Langerhans islets by vital staining by reagents: The practically largest dosis of a diabetogenic reagent was injected through the auricular, the femoral or the tail veins of rabbits and mice, which were sacrificed 5, 10, 20~30, 60 or 180 minutes thereafter. These sacrificed animals and those who died following the injection were immediately laparotomied and small portions of their pancreas cut out with scissors, placed on object glasses and covered with decking glasses, pressed lightly into thin layers and examined under a microscope without delay, to examine whether and how they are stained.

The methods for measuring the sugar in blood and urine and of histological examination adopted in such experiments were as follows:

(a) For measurement of blood sugar: Blood extracted from the auricular veins tested by Hagedorn-Jensen's method.

(b) For measurement of urinary sugar: Benedict's reagent was used.

6 drops of the tested urine were added to 3 cc. of the reagent and the mixture boiled for 2 minutes and cooled. The results were classified and marked as follows:

Solution bluish green without precipitation: ±
(Content of sugar in urine less than 0.02%).
Sol. bluish green with yellowish-blue precip.: +
(Content of s. in u. ca. 0.02~0.2%).
Sol. bluish green with red-brown precip.: ++
(Content of s. in u. ca. 0.2~0.5%).
Sol. green with reddish-brown precip.: +++
(Content of s. in u. ca. 0.5~1.5%).
Sol. colorless with red precip.: +++
(Content of s. in u. more than 1.5%).

(c) For determination of aceton-compounds in urine: Legal's test method.

(d) For histological examination: The routine staining methods used in histological researches. Langerhans islets specially examined under Gomori's staining.[19]

The samples were taken from the three portions of the pancreas, i.e., its duodenal, lienal and the intermediate parts. These tissue samples were divided into small pieces and examined separately. The number of α- and β- cells under Gomori's staining in 20~50 islets per portion was counted and the average taken as the mean number of α- and β-cells per islet.

Results

With quinoline compounds: 41 varieties of quinoline compounds were examined, always used intravenously. Seeing that oxine showed strong diabetogenesis in summer, when the room temperature exceeded 20°C, and weak efficacy in winter (room temperature below 10°C),[6] we took care to keep the room temperature between 20°C and 33°C for some hours before and after the administration or even longer.

The research revealed that 15 of these compounds are diabetogenic and the minimum required quantity for inducing the illness by them has been determined (Table I). In the other 26 we could not observe diabetogenesis (Table II), but 5-methyl-8-hydroxyquinoline and 8-hydroxy-2-(α-methyl)-quinolinemethanol proved β-cell-injurious, reducing the cell number to $\frac{1}{2}$ or $\frac{2}{3}$ of the normal in doses of 30 mg./kg. and 100 mg./kg. respectively, so that we may well suppose that diabetes might be induced, if larger doses could be administered. Prof. Y. Kotake[20,21] has asserted the diabetogenic effect of xanthurenic acid upon experiments with white rats, but we could not find any such effect with this chemical, either symptomatically or histologically. The samples of the reagent used by Prof. Kotake were isolated from urine of white rats and that used by us were artificial synthetic preparations (two brands with m.p. at 286°C and 295°C), and on this a discussion was held between Kotake and Okamoto at a biochemists' meeting.[13] Abe[22] also denies the diabetogenesis of this reagent (synthetic) in his report.

Upon examining the 15 newly discovered diabetogenic reagents, we see that without exception they are substances reading and sensitively apt to combine with zinc, but there is a considerable difference among them in the minimum amount required for inducing diabetes. To wit, (A) 5-(phenylazo)-8-hydroxyquinaldine, 5-(*p*-dimethylaminophenylazo)-8-hydroxyquinaldine, 5-(*p*-acetaminophenylazo)-8-hydroxyquinoline, and 5-(*p*-acetaminophenylazo)-8-hydroxyquinaldine require only 5 mg./kg., (B) 8-hydroxyquinaldine and 5-(*p*-aminophenylazo)-8-hydroxyquinoline, 10 mg./kg., (C) 5-(*p*-aminophenylazo)-8-hydroxyquinaldine, 15 mg./kg., (D) 5-(phenylazo)-8-hydroxyquinoline, 5-(*p*-tolylazo)--8-hydroxyquinoline and 5-(*p*-hydroxyphenlazo)-8-hydroxyquinoline, 20 mg./kg., (E) 5-(*m*-hydroxyphenylazo)-8-hydroxyquinoline and 5-(*p*-hydroxyphenylazo)-8-hydroxyquinaldine, 30 mg./kg., (F) 2,4-dimethyl-8-hydroxyquinoline, 35 mg./kg., (G) 5-(*o*-tolylazo)-8-hydroxyquinoline, 40 mg./kg. and (H) 5-(*p*-dimethylaminophenylazo)-8-hydroxyquinoline 45 mg./kg., respectively. These figures are all lower than the diabetogenic dosis of oxine, 50～60 mg./kg., and the dosis 5 mg./kg. of the (A) group is small indeed.

Next, we will observe the change in blood sugar content in the earliest stage following administration of diabetogenic substances. It is well known that three phases of glycemia appear upon administration of alloxan in a quantity sufficient for inducing diabetes, the first of temporary high glycemia for the first 6 hours, the next of extremely low sugar content in blood, from 6 to 12 hours and then of permanent high glycemia from 12 hours after the injection onwards, passing over into diabetic condition.[4,6] Okamoto *et al.*[5,7] have reported that the change in blood sugar content upon administration of dithizone takes nearly the same course and that the time table is more protracted with oxine, the first stage continuing to about the 12th hour, the low glycemia thereafter down to the 24th or even to the 30th hour, the permanent high glycemia setting in only in about 48 hours after the administration. The schedules of change in blood sugar contents following administration of the above 15 reagents are much variegated, some following the pattern of alloxan and dithizone, some resembling that of oxine and some others again taking entirely dissimilar courses. We can distinguish the following six types of change patterns (Fig. 1):

Type I: The first phase above is here lacking, the blood sugar content dropping immediately upon administration, showing an extreme low value in 1～2 hours (in such cases, death by cramps had to be prevented by frequent administration of glucose, i.e., about 10 injections of 1 g./kg. per hour. The same may be said of all the low blood sugar phases given below). In about 10 hours, the blood sugar content begins to rise and reaches the level of ca. 300～350 mg./dl. in about 24 hours. 5-(*p*-hydroxyphenylazo)-8-hydroxyquinoline shows the most typical curve of this type, and administration of 5-(*p*-dimethylaminophenylazo)-8-hydroxyquinoline and of a large dosis of 5-(*p*-acetaminophenylazo)-8-hydroxyquinoline (from 30 to 50 mg./kg.) also causes this type of change (A_1 A curve).

Type II: A slight rise of blood sugar of 170～180 mg./dl. appears in around 2 hours after the administration, dropping below normal in 3～4 hours,

TABLE I

Experimental Results on Diabetogenic Quinoline Compounds

Numbers of cells in islet of Langerhans of normal rabbits (means of 26 heads; body weight, 1900~3500 g.; both male and female) are as follows: α-cells . . . 13.1 (11.0~14.1), β-cells . . . 37.9 (36.0~40.2).

Name of reagents with m.p., lethal dose in mice (mg./kg.) and color in parentheses	Solution	Animal No.	Sex	Body weight(g.), at the time of administration	Dose (mg./kg.)	Duration of observation	Experimental results with rabbits: Blood sugar (mg./dl.) after administration in hours: bef. adm.	1/2	2	6	8	12	24	24 ×2	24 ×3	24 ×4	24 ×5	Persistence	Urine sugar	Glucose admin.	Presence of aceton body	Cells of Langerhans islets at the end of experiment): α-cells	β-cells	Findings	Combination with zinc
8-Hydroxyquinaldine[11] (74°C, 60, white)	Dissolved in dilute hydrochloric acid and then was prepared to make 0.5% solution with distilled water	4005	♂	2250	40	46 days	92	121	477	149	130	64	182	255	261		325	+	###	−	−	18.9	11.5	Injurious to β-cells (atrophy of β-cells, nuclear degeneration)	+
		8401	♀	2320	40	129												+	###	+	+	13.6	3.1		
		902	♂	2150	40	30 min.	73	98									398					14.9	20.7		
		904	♂	2150	40	2 hrs.	72		195										−	−	−	15.5	16.4		
		3714	♂	2000	40	12	72		178	205	204	272							###	+	−	16.2	12.8		
		3711	♂	2300	40	18	78	120	172	199	230	117	99						###	+	−	17.1	7.1		
		903	♂	2150	40	48	75		217	292		198	219	271					###	−	−	12.3	5.9		
		3715	♂	2000	40	48	76		212		224	304	282	310					###	−	−	14.5	4.6		
		901	♂	2200	40	5 days	86		187	280		310	175	259	301	289	293		###	−	−	12.7	5.7		
		3709	♂	2250	40	5	84		168	378	343	273	281	301	312	282	310		###	−	−	9.0	1.3		
		4003	♂	2080	35	74	88	83	413	246	163	128	100	277	573			+	###~###	+	+	13.5	9.3		
		4004	♂	2400	35	408	89	91	445	270	185	69	210	255	261			+	###	+	+	11.7	2.9		
		Y23	♂	2520	30	60	96		288	298	218	63	113	78	279	375		+	###	+	−				
		4002	♂	2040	30	492	85	85	174	89	74	53	128	302	618			+	###	+	−				
		4011	♂	2080	20	7												+	###~+	+	!				
		4006	♂	2700	20	310	112		226	230	186	215						+	###	+	−	10.9	3·0		

		4008	♂	2300	15	7	98	136	308	485	219	98	316					卌~−	+	−				
		4010	♀	2500	10	16												卌	+	−				
		8400	♀	2700	10	18												卌~−	−	−				
		4007	♂	2500	10	120	102		313	194	161	111	330				+	卌	+	−				
		4009	♀	2000	5	10	107	177	313	293	281	161						卌~−	+	−				
		4012	♂	2800	5	8	91	144	294	264	254	146						卌	+	−				
5-Phenylazo-8-hydroxyquinoline[11] (175~176°C, 25, dark-brown)	Used as 0.1% solution in hot 0.1% NaOH aqueous solution	4020	♀	2000	25	4 hrs.													−	−	14.2	12.7		
		4012	♂	2200	25	12													+	−	12.5	3.9		
		4021	♂	2300	25	24													+	−				
		4024	♂	2600	25	69 days	82	264		287		348				402	+	卌	+	+	8.4	5.2		
		5553	♀	3000	25	1.3 hrs.	92	234										−	−	−				
		3722	♂	2600	25	27 days	75		108	157		266		217		225	+	卌	−	−	8.3	3.9	″	+
		4023	♂	2200	20	20			264	335	348	128						±~卌	+	−	14.8	18.3		
		4013	♂	2200	20	7	91				62	128	256			272		卌	−	−	14.6	7.0		
		4022	♂	1950	15	4			171									卌~−	+	−	10.6	27.1		
		4035	♀	3100	15	3				88								卌~−	−	−				
5-Phenylazo-8-hydroxyquinaldine[11] (105°C, 30, violet-brown)	Used as 0.05% solution in hot 0.1% NaOH aqueous solution	4030	♂	2400	30	2 hrs.													−	−	13.2	15.4		
		1710	♂	2000	20	″													−	−	13.8	18.1		
		4031	♂	2500	20	2 days	92		264								+		−	−	14.1	12.5		
		Y111	♂	2050	20	10	87		181			230					+	卌	+	−	13.1	4.0		
		4033	♂	2650	15	146	75		172		165	216	311	268		280	+	卌	+	+				
		3724	♂	2540	15	241	84		161			189	153	230	285	300	+	卌	+	−			″	+
		1706	♂	2060	10	30 min.	77	86											−	−				
		1709	♀	2360	10	12 hrs.	78		286	50	39								−	−	18.0	7.3		
		1707	♂	2050	10	24	79		193	239	83								−	−	9.8	5.9		
		1705	♂	2050	10	2 days	96		296	254	236	30	340						−	−	14.7	3.2		
		1708	♂	2100	10	5	87		304	270	186	194	358	268	233	260	+	卌	−	−	14.4	4.3		

Name of reagents with m.p., lethal dose in mice (mg./kg.) and color in parenthèses	Solution	Experimental results with rabbits: Animal No.	Sex	Body weight (g.), at the time of administration	Dose (mg./kg.)	Duration of observation	Blood sugar (mg./dl.): bef. adm.	after administration in hours: 1/2	2	6	8	12	24	24 ×2	24 ×3	24 ×4	24 ×5	Persistence	Urine sugar	Glucose administr.	Presence of aceton body	Cells of Langerhans islets the end of experiment): α-cells	β-cells	Findings	Combination with zinc
5-(*p*-Tolylazo)-8-hydroxyquinoline[II] (191°C, 90, orange-brown)	0.1% sol. in hot 0.1% NaOH aq. sol.	4032	♂	2100	10	561 days	86		264	120	86		298	310	330	342	280	+	+++	+	−	12.6	4.7	"	+
		3728	♂	3040	10	21	71		195									+	+++	+	−	12.3	4.2		
		4037	♂	2050	5	14	75					88	96	318				+	+++	+	+				
		4036	♂	2350	5	781	77		266	242		127	56	138	163	296	302	+	+++～−	+	−	12.6	13.7	"	+
		4038	♂	2030	3	20			182	186		254	260						+～+++	−	−				
		4039	♂	3000	2	3			233	245		185	125	129					±～+	−	−				
2, 4 - Dimethyl - 8 - -hydroxyquinoline[II] (65°C, 50, white)	0.25% aq. sol. after dissolved in dil. HCl	4053	♂	2000	40	4 hrs.	86	236	375	(Died of hypoglycemic convulsion)									+	−	12.3	18.2	"	+
		4049	♂	2000	40	7 days	78	145	286	66		51	67	237	312		253	+	+++	+	±	15.5	5.2		
		5557	♂	3050	40	315	93	200	257	70		45	75		325		295	+	+++	+	−	14.5	4.8	"	+
		5558	♂	2300	35	12		243	376	361		335	526	343	287			+	+++～−	−	−				
		4044	♀	2750	30	10		126	257	272		216	207	258					+++～−						
		4042	♂	2300	20	5		199	296	211		196	170	185					+++～−						
		3716	♂	2300	30	97	88		192	119		235	252				304	+	+++	+	−			"	+
		3778	♂	2000	30	350	90		201	80		305						+	+++	+	−				
		Y41	♂	2300	20	3																			
		3729	♂	2000	20	22																			
		9001	♂	2400	20	42	102		188	85		94	296	311			286	+	+++	−	+			"	+
		4035	♂	2000	20	179																			
		4043	♂	2110	15	14	78		163	169		103	165	344			282	+	+++	−	−	12.2	4.4		
		4045	♀	2080	10	12		128	272	292		243	197	239					+++～−						

5-(o-Tolylazo)-X[12] (181°C, yellow-brown)	0.1% in hot 0.1% NaOH	3723	♂	2420	40	12 hrs.	73		75	67		44	(Died of hypoglycemic convulsion)							—	—	17.1	5.5	〃	+
		3784	♂	2200	40	192 days	92		186		56		236	234			280	+	卌	+	—	11.9	5.0		
5-(m-Hydroxyphenylazo)-X[12] (190~194°C, 100, brown)	1% in hot 0.5% NaOH	3304	♂	2350	50	111	85		170	58	93		215	266			312	+	卌	+	+			〃	+
		3707	♂	2300	40	4	75	183	256	313		218	229	226					卌~—	—	—				
5-(p-Hydroxyphenylazo)-8-hydroxyquinoline[12] (234~235°C, 150, light yellow)	0.5~0.1% sol. in hot 0.5% NaOH aq. sol.	3754	♀	2200	50	2 hrs.	105	83	17	(Died of hypoglycemic convulsion)										—	—	11.7	8.3		
		3725	♂	2210	50	5 days	77		14				159	380	358	364	387	+	卌	+	‡	13.6	3.0		
		3204	♂	2020	40	1.5 hrs.	94	72	(Died of hypoglycemic convulsion)										—			11.1	13.5		
		3208	♀	2600	40	4	74	50	73	78									—	+	—	13.6	5.5		
		3207	♀	2800	40	6	100	69	30	109									—	+	—	13.9	3.4		
		3205	♂	2020	40	12	89		36			86							—	+	—	14.3	3.9		
		3206	♂	2150	40	24	83		33			101	385						卌	+	—	12.8	3.3		
		3203	♂	2000	40	48	84					170	339	385					卌	+	—	12.9	2.8	〃	+
		3726	♂	2200	40	450 days (Recovery)	75						168	360				+	卌	+	±				
		3750	♂	1950	40	Persisting	95		39	82		263	376					+	卌	+	+				
		3751	♂	2650	30	711 (Recovery)	100	62	48	284		369	384				350	+	卌	+	—				
		3732	♂	2250	20	48 hrs.			234	270		237							卌	—	—				
		3739	♀	2520	20	10 days													卌	—	—				
		3199	♂	2100	10	7													卌~—	—	—				
5-(p-Hydroxyphenylazo)-8-hydroxyquinaldine[14] (172°C, 120, orange-brown)	0.5% sol. in hot 0.5% NaOH aq. sol.	3738	♂	2050	60	8	78	136	330	331		63	70	350			403	+	卌	—	—	13.1	4.1		
		3734	♂	2100	40	48 hrs.	105		316	270		152	254					+	卌	—	—	12.6	3.0		
		3737	♂	2000	40	260 days	91		273	307		176	198	321			358	+	卌	—	—				
		3745	♂	1900	30	28	101		276	302		279	233	311	380			+	卌	+	—	11.1	8.7	〃	+
		3733	♂	2800	20	9 (Recovery)		167	300	341		216	209	233					卌~—	—	—				
		3735	♂	2020	10	3 (Recovery)	90		179	300		206	276	201	132				卌~—	—	—				

Experimental results with rabbits

Name of reagents with m.p., lethal dose in mice (mg./kg.) and color in parentheses	Solution	Animal No.	Sex	Body weight (g.) at the time of administration	Dose (mg./kg.)	Duration of observation	Blood sugar (mg./dl.) bef. adm.	after administration in hours 1/2	2	6	8	12	24	24 ×2	24 ×3	24 ×4	24 ×5	Persistence	Urine sugar	Glucose administr.	Presence of aceton body	Cells of Langerhans islets (at the end of experiment) a-cells	β-cells	Findings	Combination with zinc
5 - (*p* - Acetaminophenylazo) - 8-hydroxyquinoline[14] (262°C, 50, yellow-brown)	0.5% sol. in hot 0.5 % NaOH aq. sol.	3755	♂	2100	50	40 hrs.	112		100	84			121	327					±	+	−	11.1	7.9		
		3758	♂	2650	30	6 days	107	53					233	367	321		349	+	###	+	−	5.7	6.1		
5-(*p*-Dimethylaminophenylazo)-8-hydroxyquinaldine[14] (175~177°C, 60, chocolate brown)	0.5% aq. sol. after dissolved in dil. HCl	3760	♀	2600	15	169	115	159	57	73		145	154	241	304		360	+	###	+	−	15.1	10.3	〃	+
		3761	♂	2000	10	54	70	107	95	62		60	199	206	314		264	+	###	+	−				
		3762	♂	2090	5	126	88	120	195	176		143	120	125					###~—	−	−	16.1	11.3		
5-(*p*-Dimethylaminophenylazo)-8-hydroxyquinoline[14] (196~197°C, 95, light brown)	0.25 % aq. sol. after dissolved in dil. HCl	3740	♂	2620	70	2	97	95	60	47		71	385	385					###	+	−	11.6	5.0		+
		3744	♂	2700	60	5 min.	72													−	−				
		3743	♂	2320	50	1 〃	88													+	−	15.9	29.8	〃	
		3779	♂	2250	45	3 days	115	10	19			47	209	256	130	(Died)			###~—	−	−				
		3991	♂	2000	40	3													###~—	−	−				
		3741	♀	2510	60	8 hrs.	109		144	35									−	−	−	15.2	4.7		
		3743	♂	2500	40	141 days	79		132	210		41	88	319	283		303	+	###	+	−	14.8	3.0		
		3746	♂	2450	30	15 hrs.	99	124	75			286						+	###	+	−	7.8	3.9	〃	+
		3742	♂	2100	20	122 days	103		287	381		297	330	321	302	380↑	433	+	###	+	+	10.1	3.2		
		3747	♂	2160	15	77	96	223	194		104		350	395	352	392	410	+	###	+	+				
		3748	♂	2030	10	25	95	283	148	74	65		160	392	380↑		388	+	###	+	−				
		3752	♂	2430	5	18 hrs.	86	90	256	231		198	(Died of hypoglycemic convulsion)					−	−	−	−	16.8	5.2		
		3754	♂	2200	5	7 days	91		216	143		126	66	264	422	417	365	+	###	−	−				
		3757	♀	2300	5	20	77		201	200		130	85	185	325	308	309	+	###	−	−	21.1	6.4		
		3753	♂	2220	3	3	105				333	210	160	169	166			−	###~—	−	−	6.5	7.8		

		3791	♂	2200	5	5	88	102	195	105	134	140	131					卌~−	−	−				
		3780	♂	2300	3	7	84	129	156	385↑	118	79	156	72				卌~−	−	−	13.3	32.8		
5-(*p*-Acetaminophenylazo) - 8 - -hydroxyquinaldine[14] (228 ~ 230°C, 60, yellow-brown)	0.04% sol. in hot 0.5% NaOH aq. sol.	3764	♀	2000	10	15 min.													−	−	12.3	22.8		
		3765	♂	2300	5	2 days	95	167	185	195	110	139	106	113					−	−				
		14008	♀	2700	5	3	77	157	182	149	153	132	140	203					−	−	15.0	11.5	″	+
		3790	♀	2400	5	35	74	198	325	258	267	247	126	167			+	卌	−	−				
		3781	♂	2250	3	16	89	129	182	153	128	103	132	82				±~−	−	−	8.5	30.8		
5-(*p*-Aminophenylazo) - 8 - hydroxyquinoline[14] (209°C, 110, yellow-brown)	0.5% sol. in hot 0.5% NaOH aq. sol.	3771	♂	2800	20	20 min.												−	−	−	10.6	22.1		
		3772	♂	2500	10	30 days	105	284	268	255	120	74	170	144			+	++~卌	−	−				
		3782	♀	3050	10	10 (Recovery)	102	372	385↑	223	372	172	208	257			+	卌~−	−	−			″	+
		4202	♀	3050	10	3 (Recovery)	71	239	269	173	189	182	196	215	(Sacrificing)			卌~−	−	−	17.5	9.9		
		15004	♀	3000	5	10	91	158	251	171	98	200	131						−	−				
		3793	♂	2200	5	2	70			138	100								−	−				
5-(*p*-Aminophenylazo) - 8 - hydroxyquinaldine[14] (156°C, 50, chocolate brown	″	4301	♂	2100	50	4	94	214	388	385				392	(Sacrificing)		+	卌	−	−	11.6	4.2		
		3767	♂	2000	45	11	108	209	385	370	385↑	298	270	305	385↑	287	+	卌	−	−				
		3770	♂	2200	30	5	92	135	385	385↑	385↑	313	164	293		212	+	卌	−	−	12.3	9.3	″	+
		3783	♂	2200	15	395 (Recovery)	107		382	280	270	300	254			325	+	卌	−	−				
		3773	♂	2100	15	7	103	158	328	385↑	382	247	385↑	237	319	267	+	卌	−	−	9.5	6.2		
		3774	♀	2550	5	3	113	148	129	146	124	131	106	90					−	−				

X 8-hydroxyquinoline

TABLE

Experimental Results with Non-

Reagent				Lethal dose in mice (mg./kg.)
Name	m.p. (°C)	Color	Solution	
5-Nitroso-8-hydroxyquinoline	245	Light green	0.5% in 0.5% NaOH aq. sol.	80
8-Hydroxyquinaldinic acid	219.5	Yellow	2% in 0.5% NaOH aq. sol.	300↑
5-Methyl-8-hydroxyquinoline	122~124	White (pale yellow	0.5% in dil. HCl	70
8-Hydroxyquinoline-2-aldehyde[14]	163~164	Yellow	0.5% in 0.5% NaOH aq. sol.	50
Xanthurenic acid[12,14]	286, 295§	〃	2% in 0.5% NaOH aq. sol.	1500↑
7-Jodo-8-hydroxyquinoline-5-sulfonic acid[12]		〃	2% aq. sol.	600↑
8-Methoxyquinoline[12]	(36 ~43)*	〃	2% aq. sol.	120↑
Quinaldinic acid[6]	156~157	White	2% aq. sol. (slightly warmed)	1200
5-Nitroquinaldinic acid	201~203	Pale yellow	0.5% in 0.5% NaOH aq. sol.	
8-Nitroquinaldinic acid	177	Yellow	0.5% in 0.5% NaOH aq. sol.	
Atophane[6]	220	White	2% in 0.4% NaOH aq. sol.	450
4-Hydroxy-8-methoxyquinaldine[12]	223~229	〃	0.2% warm distilled water	220↑
4, 8-Dihydroxyquinaldine[12]	276	〃	0.2% with aq. sol. after dissolved in dil. HCl	56
5-(*p*-Nitrophenylazo)-8-hydroxyquinoline[11]	281	Light brown	0.1% in hot 0.5% NaOH aq. sol.	40↑
5-(*m*, *p*-Diaminophenylazo)-8-hydroxyquinoline	232	Red-brown	0.5% in hot 0.5% NaOH aq. sol.	170
5-(*p*-Sulfophenylazo)-8-hydroxyquinoline[11]	250<	Brown	0.5%~1% sol. (sodium salt)	100
5-(*o*-Carboxyphenylazo)-8-hydroxyquinoline[11]	250<	〃	1% with aq. sol. after dissolved in 0.5% NaOH aq. sol.	75↑
5-(*p*-Arsonophenylazo)-8-hydroxyquinoline[14]	235	Orange-yellow	1% in 0.5% NaOH aq. sol.	100
5-(*p*-Bromophenylazo)-8-hydroxyquinoline[14]	224	Cadmium-yellow	0.25% in 0.25% NaOH aq. sol.	60↑
5-(*p*-Tolylazo)-8-hydroxyquinaldine	195~196	Violet-brown	2mg. in aceton 2.5cc.	70
5-(*p*-Nitrophenylazo)-8-hydroxyquinaldine	204	Dark brown	0.1% in 0.5% NaOH aq. sol.	90↑
5-(*p*-Sulfophenylazo)-8-hydroxyquinaldine[12]	250<	Brown	1% in 0.5% NaOH aq. sol	260↑
5-(*p*-Arsonophenylazo)-8-hydroxyquinaldine[14]		Dark brown	0.5% in 0.5% NaOH aq. sol.	130
Indoxine[11]	220	Violet-red	saturated sol. in aceton (1mg. in 1cc.)	7
5-(*p*-Bromophenylazo)-8-hydroxyquinaldine	164	Dark brown	0.1% in 0.5% NaOH aq. sol. (difficult to dissolution)	100↑
8-Hydroxy-2-(α-methyl)-quinolinemethanol	127 ~128	White	1% with aq. sol. after dissolved in dil. HCl	125↑

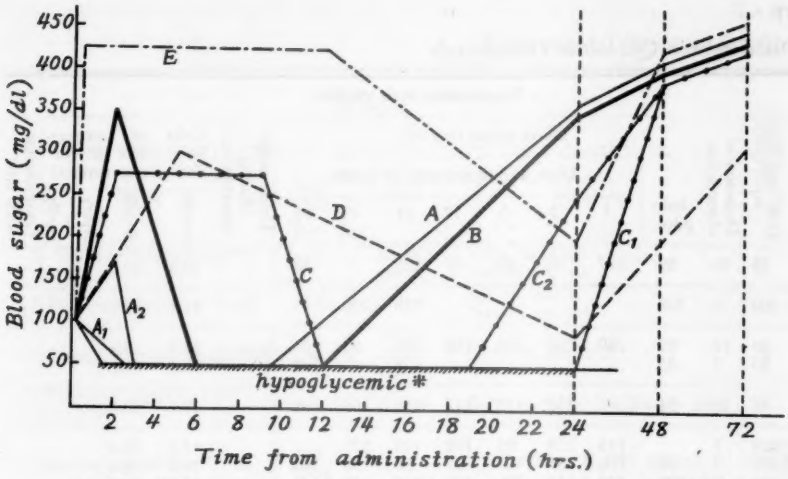
* It was moisture absorbent and difficult to measure.

II
Diabetogenic Quinoline Compounds

Experiment with rabbits

Dose (mg./kg.)	Duration of observation	Blood sugar (mg./dl.)								Urine sugar (during observation)	Cells of Langerhans islets (at the end of experiment)			Combination with zinc
		bef. adm.	After administration in hours								α-cells	β-cells	injury of β-cells	
			1	3	6	12	24	24 ×2	24 ×3					
35	10	89	137	86	81	94	126		75	–	10.3	39.0	–	+
100	5	108					118	108		–	12.1	38.2	–	+
30	10	92	280	230	205	198	185	93	93	++~–	17.3	19.9	+	+
30	7	85					168		102	++~–	16.5	21.2		
35	40	94		252	115	112	114	106	90	–	14.1	36.1	–	+
1000	7		115	128	93	102	110	121		–	14.0	35.4		
§600	3	86	166	187	107	69	86	100	103	–	14.7	33.4	–	+
500	5	105	116	116	100	116	195	98	141	–	13.8	36.0		
500	5	81	103	92	96	88	88	81		–	10.2	39.8	–	+
50	5	89					112	118		–	13.2	38.8	–	–
120	5	95	94		105	109	103	112		–	14.2	37.0	–	+
40	5	92				95	100	115		–	14.4	36.5		
100	5	85					123	115		–	13.3	39.1	–	+
100	5	95					109	106		–	14.2	38.8	–	+
ca. 1200†	7	88					91	125		–	11.9	39.8	–	+
70	2	102	169		80	99	100	112		–	16.9	36.1	–	–
200	2	105	128		112	83	81	113		–	18.9	36.1	–	–
35	8	92	154		143	107	94		120	–	17.3	37.9		
10	6	99	126	121	122	107	70	121		–	12.5	33.5	–	–
60	10	94	167	109	90	91	98	101	111	–	14.2	35.9	–	+
90	3	92	98		67	96	105			–	14.0	34.7	–	+
120	2				80					–				
38	15	103	185		114	81	112	120		–	12.8	38.5	–	+
50	2	117	133	158	146	118	125	130		–	10.2	35.2	–	+
40	2						120	118		–	15.0	34.2	–	+
6	15	101	136	110	112	124	113	115		–	13.2	37.8	–	+
10	5	95		108			112	121		–	14.0	38.2	–	–
120	12	87			103			115		–	12.8	39.3	–	+
60	5	72	77	65	58	65	65	108	143	–	8.6	36.1	–	+
3	3	90	122	110	96	73	68	93		–	10.0	40.0	–	+
40	2	93					108	115		–	8.2	33.8	–	+
100	3	74	99	95	71		98	92	90	–	9.8	25.7	+	+

† Injected on three successive days.



* Injected intravenously with 10cc. of 20 per cent glucose solution 8~10 times every one hour.

Fig. 1. Blood Sugar Curves at the Early Stage After the Administration of Diabetogenic Reagents in Rabbits.

- A
  - A_1 . . .
    - 5-(*p*-Hydroxyphenylazo)-8-hydroxyquinoline
    - 5-(*p*-Dimethylaminophenylazo)-8-hydroxyquinoline
    - 5-(*p*-Acetaminophenylazo)-8-hydroxyquinoline
  - A_2 . . .
    - 5-(*p*-(or (or *o*-) Tolylazo)-8-hydroxyquinoline
    - 5-(*m*-Hydroxyphenylazo)-8-hydroxyquinoline
    - (5-(Phenylazo)-8-hydroxyquinaldine)
- B . . . . . .
  - Alloxan
  - Dithizone
  - 2, 4-Dimethyl-8-hydroxyquinoline
  - 5-(*p*-Dimethylaminophenylazo)-8-hydroxyquinaldine
- C
  - C_1 . . .
    - Oxine
    - 5-(*p*-Hydroxyphenylazo)-8-hydroxyquinaldine
  - C_2 . . .
    - 8-Hydroxyquinaldine
    - 5-(Phenylazo)-8-hydroxyquinoline
    - 5-(Phenylazo)-8-hydroxyquinaldine
- D . . . . . .
  - 5-(*p*-Acetaminophenylazo)-8-hydroxyquinaldine
  - 5-(*p*-Aminophenylazo-8-hydroxyquinoline
- E . . . . . .
  - 5-(*p*-Aminophenylazo)-8-hydroxyquinaldine

necessitating administration of glucose, and the final high level is reached in about 10 hours. The curve of change is here similar to that of Type 1 except that there is a low peak of glycemia at the outset (A_2 A curve). 5-(*p*-tolylazo)--8-hydroxyquinoline, 5-(*o*-tolylazo)-8-hydroxyquinoline and 5-(*m*-hydroxyphenylazo)-8-hydroxyquinoline belong to this type and a similar curve appears following administration of a small dosis (15 mg./kg.) of 5-(*p*-acetaminophenylazo)-

-8-hydroxyquinoline. In most cases, the change following administration of 5--(phenylazo)-8-hydroxyquinaldine showed the pattern of CC_2 curve described below, but in only one case, the curve was of this A_2 A pattern.

Type III: The type including the curves following alloxan and dithizone administration mentioned above. In several hours after the administration a primary rise of blood sugar content appears, attaining the maximum of 200～300 mg./dl. in about 3 hours. The phase of low blood sugar content sets in about 6 hours, necessitating administration of glucose. The blood sugar reincreases from around 12 hours and rises to 300 mg./dl. or more in 24～48 hours, retaining this level thereafter (B curve). Such curves are observed upon administration of 2,4-dimethyl-8-hydroxyquinoline and 5-(*p*-dimethylaminophenylazo)-8-hydroxyquinaldine.

Type IV: Here, a primary high glycemia begins about 2 hours after the administration, keeping the level of ca. 250 mg./dl. for several hours. In 12～24 hours, low blood sugar content becomes marked, necessitating glucose injections, but in 48 hours the permanent high glycemia sets in, as was in the case of oxine (CC_1 curve). 5-(*p*-hydroxyphenylazo)-8-hydroxyquinaldine belongs to this type.

Type V: The curve in somewhat similar to the preceding Type IV curve, but here the secondary rise of blood sugar content comes a little sooner in about 20 hours after the administration (CC_2 curve). 8-hydroxyquinaldine, 5-(phenylazo)-8-hydroxyquinoline and 5-(phenylazo)-8-hydroxyquinaldine show such curves.

Type VI: In 2～3 hours after the administration, the blood sugar rises to 300～400 mg./dl., but slowly drops thereafter or after keeping that high level for ca. 10 hours, to approach the normal value and thus without showing abnormally low blood sugar content, begins to rerise in 24～48 hours (D or E curve). Such curves are observed following administration of 5-(*p*-acetaminophenylazo)-8-hydroxyquinaldine, 5-(*p*-aminophenylazo)-8-hydroxyquinoline and 5-(*p*-aminophenylazo)-8-hydroxyquinaldine.

We could not detect any correlation between the minimum doses of any reagent required for inducing diabetes and the above types of curves. With the same reagent, however, it was observed that when a dosis administered was larger than the minimum effective quantity, the low glycemia in the second phase was severe and conspicuous, but when the dosis was near the minimum effective quantity, this low glycemia was not severe nor remarkable and the primary high blood sugar phase tended to be prolonged. Upon comparing the effect of various reagents, the higher severity of diabetes following intense decrease of blood sugar content was demonstrated with clarity by blood and urine sugar tests, and difficulty of recovering and a bad reduction of β-cells were also observed. That the subsequent diabetes is severe in the case when the drop of blood sugar content was marked we have also frequently experienced in alloxan diabetes, as Goldner and Gomori[23] have reported.

Lesions in the splanchnic organs: Of course, the β-cells in the Langerhans islets are most conspicuously affected upon administration of diabetogenic

reagents. As seen in alloxan,[3,4,24,25] dithizone[6,7] and oxine[6,7] diabetes, such lesions finally lead to necrosis and disappearance of β-cells (Fig. 2~30). In a tissue section of pancreas of a rabbit 5~10 days after occurrence of diabetes, the number of β-cells per islet has fallen below 10. We have made somewhat more detailed examination after administration of 8-hydroxyquinaldine, 5--(phenylazo)-8-hydroxyquinaldine and 5-(p-hydroxyphenylazo)-8-hydroxyquinoline, and found that at first the granules of β-cells are decreased and pycnotised (the first stage), next the β-cells suffer necrosis in a wide extent and a homogenous necrosis matter is formed in the islets (the 2nd stage), and finally this necrosis matter is absorbed and eliminated, so that the islets end by consisting of almost nothing but α-cells, though sometimes a few tiny β-cells are found remaining (the 3rd stage). The speed of this degeneration of the β-cells is sharply differentiated according to the kind of the reagents used and exactly corresponds with the change in speed of the rise and fall of blood sugar content. For example, with 5-(p-hydroxyphenylazo)-8-hydroxyquinoline which causes speediest change in blood sugar content, the necrosis of β-cells is also observed the soonest, being marked from a half to 12 hours after the administration (Figs. 19, 20, 21, 22), while upon administration of 8-hydroxyquinaldine and 5--(phenylazo)-8-hydroxyquinoline tardy in causing change of blood sugar content, the β-cells fall into necrosis at the latest, beginning to be perceptible 12~24 hours after the administration (Figs. 4, 5, 6 and 9). Upon administration of alloxan[24,25] and dithizone[6,7] causing change of blood sugar content of a speed middling of the two above, the necrosis of β-cells become conspicuous in 6~12 hours. Thus, the time of conspicuous necrosis of β-cells clearly coincides with the time of the low blood sugar content when attacks of cramps are frequent.

No marked changes are observable in the organs other than the Langerhans islets, except a slight change in the heart (beleding from blood capillaries, blurring of the stria in early stage and deposition of fat).

After the lapse of 1 or 2 years from the diabetization, the islet cells become generally atrophied and the number of the islets is also reduced, but there are also found some islets that show the appearance of having been regenerated. In such a renovated islet, β-cells are in predominance, containing some very hypertrophied specimens and α-cells are nearly absent. In cases of long-standing diabetes, the pancreas is sometimes found cirrhotic and the kidneys, the liver and the adrenal cortex hypertrophied. Details on this point will be published in a separate report.

Results upon administration of nicotine:

Though nicotine is not very sensitively zinc-affine, it forms precipitates upon reacting with zinc. So, we took up this reagent and experimented with it upon many rabbits. To avoid instantaneous death and loss at appetite upon injection of nicotine, only robust rabbits with strong appetite were selected and in extremely hot and cold and rainy seasons the experiments were suspended. As cramps follow injection of nicotine, we took care to finish injection of the full dosis before the onset of the cramps, that is, within ten seconds. Clonus directly follows an injection of nicotine and then a stage of collapse. In the Groups

TABLE III

Experimental Results with Rabbits by Nicotine

Group of experiment	Number	Sex	Method and dose of administration (mg./kg., intravenously, the figures in parentheses show number of days between administrations)	Number of rabbits	Incidence of diabetes	No. of days of observation (after the last administration)	Days between last injection and incidence	Duration of diabetes	Blood sugar (mg./dl.)	Urine sugar (during observation)	Cells of Langerhans islets (at the end of experiment)		
											α-cells	β-cells	Findings
I	1	♂	0.5 —(7)→ 0.5	3	2	8 days	6	1		++			
	2	♂	0.5 —(7)→ 0.5			10 〃	6	2		+	8.1	19.5	
	3	♂	0.5 —(10)→ 0·5			14 〃	0	0		−	7.7	26.9	
II	4	♂	1	9	2	15 〃	8	1		+++～−	7.6	23.4	Vacuolation, collapse, nuclear dissolution etc. observed in β-cells.
	5	♂				10 〃	6	1		+++	14.9	16.9	
	6	♂				2 hours	0	0		−			
	7	♂				24 〃	0	0		−	13.9	17.2	
	8	♂				24 〃	0	0		−	15.9	23.4	
	9	♂				7 days	0	0		−	12.0	19.0	
	10	♂				3 〃	0	0		−	17.4	33.0	
	11	♂				4 〃	0	0		−	13.3	35.6	
	12	♂				14 〃	0	0		−			
III	13	♂	1 —(10)→ 1	5	2	15 〃	5	4	191‡ 203†	+++～++	13.1	15.1	〃
	14	♂				31 〃	4	1		+	12.5	17.5	
	15	♂				17 〃	0	0		−	11.6	25.4	
	16	♂				13 〃	0	0		−			
	17	♂				*	0	0		−	10.7	31.4	
IV	18	♂	2	6	4	8 days	3	2	213‡	+++	10.8	17.0	〃
	19	♂				11 〃	2	1		+++	11.8	16.7	
	20	♂				5 〃	2	ca. 1		++			
	21	♂				5 〃	1	1		+	13.2	21.0	
	22	♂				5 〃	0	0		−			
	23	♂				*	0	0		−	10.7	34.0	
V	24	♂	1, 2	5	3	10 days	3	1	175‡	+++	13.2	18.2	〃
	25	♂				21 〃	8	2		+++～+			
	26	♂				7 〃	3	3		++～+	12.9	18.0	
	27	♂				7 〃	0	0		−	10.2	20.8	
	28	♂				3 hours	0	0		−	10.7	31.4	

Group of experiment	Number	Sex	Method and dose of administration (mg./kg., intravenously; the figures in parentheses show number of days between administrations)	Number of rabbits	Incidence of diabetes	No. of days of observation (after the last administration)	Days between last injection and incidence	Duration of diabetes	Blood sugar (mg./dl.)	Urine sugar (during observation)	Cells of Langerhans islets (at the end of experiment)		
											α-cells	β-cells	Findings
VI	29	♂	(5~15) 1, 2 ⟶ 1, 2, 2	5	4	7 days	9	3	233§	###~+	7.8	17.3	〃
	30	♂				9 〃	6	3		###~+	8.8	19.9	
	31	♂	(5~15) ⟶ 2, 2, 2			30 〃	3	2		###~+	10.2	20.8	
	32	♂				11 〃	1	11		###	14.3	20.3	
	33	♂				1 day	0	0		–	14.3	20.3	
VII	34	♂	(10) 1, 2, 2 ⟶ 2	3	3	10 days	5	4	225#	###~##	10.1	17.8	〃
	35	♂	(10) ⟶ 2, 2, 2			18 〃	9	9		###	12.9	18.0	
	36¶	♀				387 〃	3	387	215~268	###	11.1	11.6‡	
VIII	37	♂	(7) 2, 2, 2 ⟶ 2, 2, 2	3	2	5 〃	4	5		###~##	8.5	14.3	〃
	38	♂	(10) ⟶ 2, 2, 2			10 〃	4	5		###~##	14.2	14.2	
	39	♂	(15) ⟶ 2, 2, 2			*	0	0		–	11.8	18.0	

Partial report of results published already (Okamoto *et al.*).[9,10]

‡ Number of β-cells severely vacuolated or collapsed = 3.3. # as of 2 days after incidence. † as of 3 days after incidence. § as of 5 days after incidence. ¶ Number of cells in caudal part, as of 33 days after incidence: α-cells 8.7, β-cells 7.7. * Died instantaneously.

IV, V and VI, in Table III the injection was repeated at 10~20 minutes intervals (the figures 1, 2 shows that 1 mg./kg. at first and then 10~20 minutes later 2 mg./kg. were injected). In 30 minutes, the blood sugar content shows a slight rise and the level is kept up for some hours, but usually it does not rise above 180 mg./dl., and no glycosuria is observed. And no abnormally low glycemia follows this transient rise. Of the rabbits given only 1 or 2 injections of small doses, as in groups I~V, about one third of the cases have glycemia or above 200 mg./dl. and excrete glycosuria for 1~3 days starting 3~8 days after the treatment, but the symptoms are not permanent. The β-cells in the Langerhans islets, however, are subject to change in such cases also. First, they become vacuolated and collapsed, their nuclei are dissolved and in rare cases, pycnosis is also observable (Nos. 6, 7 and 8). Such degenerations are observable even after the lapse of 10 days after the injection and the number of β-cells are reduced to less than half in a considerable number of cases.

When as large as possible doses per injection are administered thrice, as in rabbits of groups VI~VIII, the animals that survived the treatment showed

blood sugar contents higher than 200 mg./dl. and excreted glycosuria without exception, beginning 3～9 days after the injection. The symptoms persisted for 3～11 days, but No. 36 had then for 387 days and finally died unrecovered. In these animals, the β-cells were also found vacuolated, collapsed or disappeared and in No. 36, such degenerations were particularly advanced. When we laparotomized this rabbit and examined a very small portion of the pancreatic tail excised from it on the 33rd day of diabetization, we found islets with very strikingly vacuolated and collapsed β-cells (Figs. 31, 32) and islets consisting solely of α-cells, the β-cells having all disappeared (Fig. 33), coexisting therein, while a very few islets contained hypertrophied β-cells. At its death 387 days after the incidence, the necrobiotic degenerations cited above of the β-cells were almost gone, only islets consisting merely of slightly atrophied α-cells and those with atrophied β-cells commingled with such α-cells being found in coexistence (Fig. 34).

It has been reported that transient hyperglycemia was observed following nicotine administration.[26,27,28,29] In particular we may note that Mosinger *et al.*[28] have reported that when guinea pigs were injected subcutaneously with nicotine many times, a blood sugar content about double of the normal (but always below 200 mg./dl.) persisted for 17 days after the injections were discontinued. This high glycemia is generally attributed to a release of adrenalin, but Burstein and Goldenberg,[30] Hazard and Vaille[26] have expressed doubts to this assumption. On our part, we have examined the Langerhans islets following nicotine administration in detail as described above and found that the β-cells were injured, in an intensity exceeding the limit of diabetization (which is set at 11～6 β-cells per islet[8]). Thus, the diabetes induced in the rabbit No. 36 following nicotine administration must be pancreatic in nature without doubt.

Results of administration of various reagents: The results following administration of substances nearly related to diabetogenic alloxan, dithizone or nicotine and non-toxic or only weakly toxic zinc-affine substances have been summarized in Table IV. In most cases, no diabetes has been induced and little change observed in β-cells, except that with 4-methylthiouracil, diphenylcarbazide, sodium diethyldithiocarbamate and guanidine carbonate the β-cells were found consumed and decreased to $\frac{1}{3}$, $\frac{1}{2}$, $\frac{1}{2}$ and $\frac{2}{3}$ of the normal number and that with diphenylthiocarbazide diabetes was found produced. In this last case, it is probable that the chemical was oxidized in the course of preparing the injection, producing diphenylthiocarbazone (dithizone) which caused the diabetes.

Progress of experimental diabetes mellitus in rabbits: The progress of the diabetes induced in rabbits by single injections of the required doses of the reagents belonging to the quinoline family newly discovered to be diabetogenic, nicotine (injected repeatedly at intervals of several days) and the established diabetes producers alloxan, dithizone and oxine was subjected to observation. Insulin treatment was not applied as far as aceton was not excreted. Only, we took care to avoid feeding barley or potatoes containing much carbohydrates to cases with highly advanced diabetes.

TABLE IV

Experimental Results with Various Reagent

Reagent		Lethal dose in mice (mg./kg.)	Experiments with rabbits											Cells of Langerhans islets (at the end of experiment)				Combination with metals	
					Blood sugar (mg./dl.)								Urine sugar (during observation)						
						after administ. in hrs.													
Name	Solution		Dose (mg./kg.)	No. of days of observation	bef. adm.	1	3	6	12	24	24 ×2	24 ×3		α-cells	β-cells	Injury of β-cells	Diabetogenic effect on rabbits	zinc	others
Anabasin	0.2% in aq. sol.	3	1	5	85					95			–	12.1	32.3	–	–	+	
Brucine[11]	0.25% in dil. alcohol	20	10	2	92						105		–	16.1	27.5			15,16) +	15, 16) Hg, Cd, etc.
			10	7							112	108	–	10.0	37.0	±	–		
5, 5-Dibromobarbituric acid[12]	2% in aq. sol.	25	20	2	88					95	98		–					–	
			15	2	84						81		–	11.6	27.9	±	–		
5-Nitrobarbituric acid[11]	2% in warm aq. sol.	1050 ↑	400	2	102						105		–	19.2	35.0	–	–	+	K, Mg[15,16]
Sodium rhodizonate[11]	1% in aq. sol.		300	1	85					112			–	13.3	31.5	–	–	+[15]	Ba, Sr, Pb, etc.[15,16]
Mesoxalic acid	1% in aq. sol.	300 ↑	180	3	85	96	94	135	89	110	85	72	–				–	+?	
4-Methylthiouracil[9] (Methiocil)	2% in aq. sol.	800 ↑	700	5	101		189	102	72	102			++~–	14.5	17.2				
			500	2	107	274	105	105	91	89			++~–	16.0	12.6		–	?	
			200	7	98		88	118	79	93			–	12.8	34.0				
Potassium urate[8]	270mg. uric acid in 24cc. of 0.5% KOH aq. sol.	100 ↑	ca.100	2	95	107		142		82			–	12.5	38.2	–	–	15) +	
			ca.100	2	92	116	63	162	129	86			–	11.0	41.3				
Lithium urate[8]	1% uric acid in saturated lithium carbonate	200 ↑	ca.200	2	87	96		129		108	86		–	12.8	38.9	–	–	+	
			ca.200	2	93	100		135		115			–	11.3	37.8				

Theophylline[12]	1% in aq. sol.	250	200	5	92						108		–	13.2	37.5	–	–	–	
Caffeine[12]	〃	100	75	5	96						121		–	14.2	38.9	–	–	–	
Theobromine[12]	0.5% in 0.2% NaOH aq. sol.	250	200	5	88						112		–	12.0	34.4	–	–	+	
5-Bromoanthranilic acid[12]	1% in dil. NaOH sol. (under 0.5%)	800	500	3	93	215	125	93	86	100	110		±～–	12.9	33.3	–	–	±[16]	Co, Cu, Ni[16]
			300	5	99					89		103	±～–	13.4	36.7				
5-Hydroxyanthranilic acid[12]	0.5% in hot aq. sol. (60～70°C)	100↑	100	2	97					108	112		–	11.2	37.5	–	–	–	
Anthranilic acid[6]	3g. dissolved in 22cc. of 1N NaOH aq. sol.	2000↑	200	2	85						102		–	13.5	35.6	–	–	±[15,16]	Co, Ni, Cd, Cu, Pb, etc.[15,16]
			200	2	98			84		98	94		–	12.2	34.8				
Creatine[12]	1% in aq. sol.	600↑	600	5	87						104		–	14.2	37.8	–	–	–	
Creatinine[12]	1% in aq. sol.	1000↑	500	5	92						112		–	12.5	38.8	–	–	±	
DL-Phenylalanine[12]	2% in 0.2% NaOH aq. sol.	500↑	500	5	96						108		–	11.9	37.4	–	–	–	
Tyrosine[12]	2% in 0.4% NaOH aq. sol.	700↑	500	5	92						98		–	13.1	39.1	–	–	–	
Dopa[12]	1% in warm aq. sol.	500↑	200	3	95						105		–	12.3	37.9	–	–	–	
L-Leucine[12]	2% in aq. sol.	500↑	500	5	87								–	14.2	38.8	–	–	–	
Isoleucine[12]	2% in aq. sol.	500↑	500	7	92								–	13.8	38.2	–	–	–	
DL-Norleucine[12]	2% in 0.2% NaOH aq. sol.	1000↑	250	7	93								–	11.0	34.0	–	–	+	
Arginine	2% in aq. sol.	1000↑	500	7	84								–	12.5	34.1	–	–	–	
Cystine	2% in 0.5% NaOH aq. sol.	300↑	200	7	91								–	11.0	33.0	–	–	+	
Cysteine	1% in aq. sol.	500↑	500	7	96								–		38.0	–	–	–	
Thionalide[9]	1% in absolute alcohol		4	1	97								--	10.1	32.7	–	–	+	Ag, Cu, Cd, etc.[15, 16]
			17		88 (Died instantaneously)														

Reagent		Lethal dose in mice (mg./kg.)	Experiments with rabbits														Combination with metals	
					Blood sugar (mg./dl.)								Urine sugar (during observation)	Cells of Langerhans islets (at the end of experiment)				
						after administ. in hrs.												
Name	Solution		Dose (mg./kg.)	No. of days of observation	bef. adm.	1	3	6	12	24	24 ×2	24 ×3		α-cells	β-cells	Injury of β-cells	Diabetogenic effect on rabbits	zinc	others
Diphenylcarbazide[9]	0.1% in 0.05% NaOH aq. sol.		30										±～−	12.5	23.1			15, 16 +	Hg, Cu, Cd, etc.[15,16]
	Satur. sol. in satur. lithium carbonate sol. (10min. at 90°C)		20cc.	2	83	72		83		88	95		±～−	11.0	21.4	+	−		
Diphenylthiocarbazide[8]	Saturated sol. in saturated lithium carbonate aq. sol. (20min. at 90～95°C)		10cc.	15	80	143		84		74	232		###	14.1	4.2				
			20cc.	15	80	280	250	35	70	280	315		###	15.2	3.2	+	+	###[15]	Hg, Co, etc.[15]
			20cc.	15	102	291		334		206			###～−						
Sodium diethyldithiocarbamate[9]	5% in aq. sol.		500	2	95	126		83	120	215	94		+～−	9.5	21.9				
			400	2	104	89		61	73	102	106		+～−	8.4	23.5				
			200	2	74	56		51	55	146	88		−			+*	−−	15, 16 ###	Hg, Cd, Mn, Co, Cu, Pb, Fe[15,16]
			200	2	75	126		65	90	114	96		−−						
			200	3	93	93		63	128		97		−						
			200	2						158	108		−						
Rubeanic acid[9]	0.5% in absolute alcohol		4	2									+～−	15.2	44.2				
			2	2	98	138				102	113		−			−	−	+	Co, Ni, Cu, Hg[15,16]
			1	2									−						
Bismuthiol I	0.5% in 0.4% NaOH aq. sol.	400	100										−	14.2	39.2	−	−	+	Bi, Cu, etc.[15, 16]
4-Hydroxybenzthiazole[6]	dissolved in dil. HCl		60	5									−−	12.1	38.2	−	−	###[16]	Ni, Cu[16]

2-Benzothiazolethiol[11] (Vulkacitmercapto)	0.5% in 0.25% NaHO aq. sol.	100	150	2									–	11.2	34.6	–	--	++[16]	Cu, Ag[16]
			100	2									–	9.7	38.0				
Benzothiazole[11]	2% warm aq. sol.	700	300	3									++~–	12.7	40.9	–	–	++[16]	Cu, Ag[16]
Benzimidazole[11]	0.8% in dil. alcohol	50	80	2										10.0	38.0	–	–	++[16]	Co, Hg, Cu, Cd[16]
Salicylaldoxime[11]	1% in dil. alcohol	120	100	2									–	13.7	39.8	–	--	+[16]	Cu, Ni, Co, Fe, etc.[15,16]
			100	2									–	9.0	39.4				
			100	2									–	16.2	35.2				
Morin[11]	2% in 0.5% NaOH aq. sol.	250	200	2									±~–	12.9	36.2	–	–	+[16]	Al, etc.[15,16]
			200	2									–	10.8	35.6				
Cholic acid (sodium salt)[11]	2% in aq. sol.	400	150	3								90	---	12.1	37.5	–	–	++	
o-Phenylenbiguanide[11]	0.2% warm aq. sol.	55	55	9				112					–	12.6	38.5	–	–	++[16]	Cu, Hg, Ag, etc.[16]
			50	1	80	83		81	90				–	13.8	39.2				
Guanidine carbonate[11]	5% aq. sol.	50	300	1	83	85		91	83	80			–	13.3	31.5				
			210	2.5 hrs.									–	12.3	26.7	±	–	++[16]	Cd, Mn, Mg[16]
			180	6 hrs.				91					–	16.7	26.2				
Ethylendiaminotetracetic acid	2% aq. sol.		150	10	106	124	89	93		74			–	13.2	37.3	–	--	?	Alkaline earth metals[17]
o-Phenanthrolin hydrochloricum[11]	0.2% aq. sol.	15	20	5									++~–	12.3	35.8	–	–	+[16]	Cr, Cu, Fe, etc.[16]
Nitrophenolarsinic acid	2% in warm aq. sol.	110	150	1									–	14.7	34.0	–	–	?[16]	Cd, Pb, Bi, Hg, etc.[15,16]
			100	2.5 hrs.									–						
Chloranilic acid	0.5% in 0.5% NaOH aq. sol.		100	2		102	106	125	120	95	84		–	12.1	37.3	–	–	+[18]	Ca, Ba, Sr

* Injurious to α-cells, too.

With oxine, 5-(*p*-acetaminophenylazo)-8-hydroxyquinaldine, 5-(*p*-aminophenylazo)-8-hydroxyquinoline, 5-(*p*-aminophenylazo)-8-hydroxyquinaldine and nicotine, the diabetes mostly alleviated and the rabbits recovered in the early stage. This is due to the lighter lesions of the β-cells in comparisons with other diabetogenic reagents (as shown in Tables I and III excepting oxine), permitting regeneration of β-cells with the lapse of time.

We will here summarize the results of administration of 14 reagents other than the 5 above (Table V). The total number of rabbits used in this experiment was 170, but these 21 cases could not be adequately observed owing to instantaneous or sudden death or death on account of excessive low blood sugar, soon after the administration, and 28 were sacrificed for histological examination in the course of the progress. Of the remaining 121, 29 got better and recovered and 92 died while diabetic. The approximate ratio of 3 : 1 of deaths and recoveries is maintained at any period, say on the 100th day, one, two or three years after the diabetization, that is throughout the entire course of the illness.

Both death and recovery are found frequent in the first 2 months, especially in the first month after the diabetization, becoming less frequent thereafter. 33% of the total cases died or recovered within 20 days, 45% within 60 days and about 50% within 100 days after the diabetization. Thus only a half of the total remain diabetic beyond the 100th day. The cases of continuous diabetes persisting over one year represented about 20% of the total and those over 2 years about 3.3%. The longest case of continuous diabetes lasted for 1100 days or about 3 years. The life-span of rabbits is known to be above 5 years, and seeing that the rabbits used in our experiments were about 0.5～1 year in age at the outset, it is clear that the rabbits made diabetic and left untreated enjoy only a shortened life.

The causes of death in 98 total deaths of the diabetic rabbits were as follows : Diarrhea (in most cases accompanying acute enteritis) accounted for 34 cases, 24 cases had severe lipemia, suppurative diseases accounted for 20 cases, general debilitation (decreased appetite leading to gradual emaciation and death) for 7 cases, want of appetite (appetite completely suppressed leading to sudden death) for 5 cases, ileus for 6 cases and other miscellaneous causes for 5 cases. So the diseases of the digestive tract, including acute enteritis and ileus lead the list occupying 40% of the total, followed by death on account of lipemia, suppurative diseases and debilitation in the order named. The suppurative diseases had their foci in the liver and the lungs in the majority of the cases, next in the pancreas, peritonitis and pericarditis being also observable.

Of the cases with lipemia a very large number died within 20 days after diabetization, most of the cases with suppurative diseases within 2 months and the majority of the cases that died of marasm survived more than 100 days. Deaths by diarrhea occurred at almost any time during the progress of the illness.

According to the results compiled from past reports by Root,[31] in the mortality statistics of human diabetes patients, in the preinsulin days, coma topped

the list, followed by cardio-renal-vascular diseases, and acute infection took also a considerable toll, but since insulin has come into use, a very large percentage is ascribed to cardio-renal-vascular diseases, the next leading cause being acute infection. Comparing these findings with those of rabbits cited above, we find that while very many of the human diabetes patients succumb to cardio-renal-vascular diseases, not one case of such a disease was found among our rabbits, while death due to diarrhea, so prevalent among the rabbits, is nearly unknown in human diabetes. How are such differences to be accounted for? This question seems to call for further researches.

Vital staining of Langerhans islets by diabetogenic reagents:

The diabetogenic reagents used in our experiments comprise many pigments. This is due to the fact that we have deliberately chosen such pigments produced by combining diabetogenic reagents such as oxine and 8-hydroxyquinaldine with azo compounds, so that we can trace more accurately the movement of the reagents in the body by their colorization. Most of these diabetogenics come into combination with the cells in the Langerhans islets upon injection and stain them distinctly, so that they appear as minute granules. Such a staining was found already with dithizone and we have reported on it in 1949.[6] In 1952 and 1954, Wolff *et al.*[32,33] have reported on the same subject with dithizone, but they seem to have been unaware of our preceding report and make no reference to it.

As seen in Table VI, we found 10 cases of positive results in vital staining following use of 13 varieties of phenylazo compounds of quinoline, among which 5-(phenylazo)-8-hydroxyquinoline, 5-(*p*-hydroxyphenylazo)-8-hydroxyquinoline, 5-(*m*-hydroxyphenylazo)-8-hydroxyquinoline and 5-(*p*-acetaminophenylazo)-8--hydroxyquinoline gave especially beautiful staining. It must be noted that whereas these reagents are red, carmine or dark carmine in solution, they stain the Langerhans islets orange-yellow or orange, and we are reminded of the orange-yellow and orange color of the zinc compounds of these reagents. The same phenomenon has been observed with dithizone also, which is red in solution, but the vital staining it produces on Langerhans islets is strawberrycolored (purple-red), as is the zinc-dithizone compound. The vital staining with quinoline-azo compounds is clearly observable in 5 minutes after the injection and attains the maximum intensity in 20～30 minutes. Thereafter, it begins to fade gradually, becoming almost imperceptible in 3 hours, as was precisely the case with dithizone.

As remarked above, some of the azo compounds of quinoline derivatives showed intense vital staining effect, while other chemicals of the same family showed weak and even in a few cases no staining power at all. This difference seems to be due to the doses of administration, the color tint of the compound of the pigment and the zinc (the difference of its visibility under a microscope) and the quantity of the pigment coming into combination with Langerhans islets. When dithizone was used, some other organs than pancreas, such as the cells of the submaxillary glands, the basal cells of the intestinal glands and their lumina and the epithelial cells of the prostata, were also found sometimes vitally

TABLE

Progress of and Causes of Death in Diabetes with Alloxan, Dithi-

The figures in brackets show the number of days of duration of urine sugar. The abbreviations in parentheses show the main causes of death: (A)=anorexia, (Acc)=accident, (Coc)=coccidiosis, (Dia)=diarrhea, (F. Li)=severe fatty degeneration of the liver, (H.C.)=hypoglycemic convulsion, (I)=incontinence of the feces and urine, (I.D.)=instantaneous

Name and dose of (mg./kg., intrave. inj.) reagent		Grand total	Total	0 ≀ 24 hrs.	1 day ≀ 4	5 ≀ 10	11 ≀ 20	21 ≀ 40	41 ≀ 60
Alloxan 200	Died while diabetes persisted	32	26	5 (I.D)···3 (H.C)···2	3 (I)···1 (Lip)···1 (A)···1	5 (Dia) ···3 (Coc) ···1 (Acc) ···1			4 (Ile)···1 (Li.A)···1 (Pneu) ···1 (Dia) ···1
	Recovery		6				2	1	
	Diabetes persisting		0						
Dithizone 100~200	Died while diabetes persisted	51	35	5 (I.D)	2 (Dia)···1 (Lip)···1	3 (Lip)···2 (A) ···1	5 (Lip)···2 (Dia)···2 (Pneu) ···1		
	Recovery		16				3		3
	Diabetes persisting		0						
Oxine 50~60	Died while diabetes persisted	18	6	4 (H.C)			1 (Dia)		
	Recovery		9		4 (N-D)···1	2	2		
	Diabetes persisting		3			2 (S)			
Nicotine 12~26	Died while diabetes persisted	11	4			2 (Dia) ···1 (A) ···1	1 (Dia)		
	Recovery		7	2 (N−D)	4	1			

V

zone, Oxine, Nicotine and Quinoline Compounds in Rabbits

death, (Ile)=intestinal obstruction, (K.A)=abscess of the kidney, (Li. A)=abscess of the liver, (Lip)=lipemia, (Lu. A)=abscess of the lung, (M)=marasm, (N-D)=not diabetic, (P.A)=abscess of the pancreas, (Pneu)=pneumonia, (P. Pc)=pyogenic pericarditis, (P.O)=pyogenic inflammation of the testicle, (P. Pt)=pyogenic peritonitis, (S)=sacrificing.

61 ≀ 80	81 ≀ 100	101 ≀ 200	201 ≀ 300	301 ≀ 400	401 ≀ 500	501 ≀ 600	601 ≀ 700	701 ≀ 800	801 ≀ 900	901 ≀ 1000	1001 ≀ 1100
1 (P. Pt)		4 (Dia)···1 (Lip)···1 (Pneu) ···1 (Lu. A) ···1	1 (Lip)	1 [359] (Lip)		1 [581] (Li.A)	1 [616] (Dia) + (Lu.A)				
		1	1	1							
1 (Dia)	1 (Dia)	2 (Lip)···1 (F.Li)···1	4 (Lip)···3 (Ile)···1	4 [400,400, 365,373] (P.Pt)···1 (Lip) + (P. Pc) ···1 (Dia)···1 (K.A) + (Li.A)···1	3 [405, [411,434] (Acc)···1 (Dia)···1 (P. Pt) + (Lu. A) ···1		3 [635, 635, 625] (M)···2 (Lip) ···1		1 [850] (M)		1 [1100] (Dia)
2	1	1	2	1	2		1				
		1 (K.A) + (Li.A)									
	1										
		1 (S)									
				1 [387] (Dia)							

Name and dose of (mg./kg., intrave. inj.) reagent			Grand total	Total	0 ~ 24 hrs.	1 day ~ 4	5 ~ 10	11 ~ 20	21 ~ 40	41 ~ 60
Quinoline compounds	‡ a	Died while diabetes persisted	87	50	11 (H.C)···9 (I.D) ···2	4 (Lip)···2 (A) ···2	5 (Lip)···3 (Dia)···2	3 (Lip)···2 (Ile) ···1	4 (P.O)···1 (Dia) ···2 (Ile) ···1	3 (Ile)···1 (Dia)···2
		Recovery		7	1 (N-D)		2	1		
		Diabetes persisting		30	14 (S)	6 (S)	5 (S)	2 (S)		1 (S)
	† b	Died while diabetes persisted	13	4	2 (I.D)		2 (Dia) ···1 (Lip)···1			
		Recovery		8	2 (N-D)	1	1	1	2	
		Diabetes persisting		1		1 (S)				

* as of March 31, 1954. ‡ 8-Hydroxyquinaldine (10~40), 2, 4-Dimethyl-8-hydroxyquinoline (40), 5-Phenylazo-8-hydroxyquinoline (20~25), 5-Phenylazo-8-hydroxyquinaldine (5~30), 5-(*p*-(or *o*-)Tolylazo)-8-hydroxyquinoline (20~40), 5-(*p*-(or *m*-)Hydroxyphenylazo)--8-hydroxyquinoline (30 (40)~50), 5-(*p*-Hydroxyphenylazo)-8-hydroxyquinaldine (30~60),

stained lightly,[6,8] but when quinoline derivatives were used, no vital staining of any nonpancreatic organs, not excluding the above cited cells, was ever found. This can be interpreted as follows. The above three oragns have been histochemically demonstrated to contain zinc, as do the Langerhans islets, but the zinc content of the former is smaller than in the latter and show considerable fluctuation in its value,[1,2,8] so that only such zinc reagents that are very sensitive and the staining they cause is intense and discernible under a microscope will produce perceptible staining in such organs, and while dithizone is one of such strong reagents, the quinoline-azo-derivatives are not so sensitive and strong--staining.

From the above findings, it has been demonstrated that the many diabetogenic reagents cited above show strong preferential affinity to the cells of the Langerhans islets, while they are in circulation, and we may conclude that this affinity is chemical in nature derived from the strong affinity of zinc and zinc reagents, from the coloration of the vital staining and the presence of zinc readily combining with zinc reagents in the islet cells. From this, we deduce that also the other diabetogenic reagents, including oxine, 8-hydroxyquinaldine and even

61 ~ 80	81 ~ 100	101 ~ 200	201 ~ 300	301 ~ 400	401 ~ 500	501 ~ 600	601 ~ 700	701 ~ 800	801 ~ 900	901 ~ 1000	1001 ~ 1100
3 (L.A) ···1 (P.A) ···1 (Lip) + (Ile) ···1	1 (Dia)	8 (Dia)···4 (M) ···1 (Li.A) ···2 (P.A)···1	2 (Dia) ···1 (M) ···1	3 [301, 315, 350] (Dia) ···1 (M) ···1 (Li.A)···1	2 [408,492] (M) ···1 (Dia) ···1	1 [561] (Dia)					
					1 [450]			2 [781, 711]			
		1 (S)						1* [742]			
				1 [395]							

5-(*p*-Dimethylaminophenylazo)-8-hydroxyquinoline (45~70), 5-(*p*-Dimethylaminophenylazo)-8-hydroxyquinaldine (5~60), 5-(*p*-Acetaminophenylazo)-8-hydroxyquinoline (5~50).
† 5-(*p*-Acetaminophenylazo)-8-hydroxyquinaldine (3~10), 5-(*p*-Aminophenylazo)-8-hydroxyquinoline (10~20), 5-(*p*-Aminophenylazo)-8-hydroxyquinaldine (15~50).

alloxan, upon administration, combine with the β-cells in quantity with preference, and that thanks to the chemical affinity of zinc and zinc reagents. Then they manifest their toxicity and kill off the β-cells. This deduction in perfectly consistent with the zinc theory of diabetes proposed by Okamoto, and is of course not agreeable with the glutathione theory on the mechanism of diabetization of Lazarow.[34]

Discussion

We will attempt some theoretic considerations in connection with the above described results of our experiments.

First, we will see what constitutes diabetogenic efficacy. Upon investigating the effects of sundry compounds, we have found that 8-hydroxyquinaldine, 2, 4-dimethyl-8-hydroxyquinoline, compounds of oxine and 8-hydroxyquinaldine with phenylazo bases affixed at its position 5, nicotine and diphenylthiocarbazide selectively cause lesions in β-cells and produce diabetes mellitus, while 5-methyl-8-hydroxyquinoline, 8-hydroxy-2-(α-methyl)quinolinemethanol, 4-methylthiouracil, diphenylcarbazide, sodium diethyldithiocarbamate and guanidine car-

TABLE VI

Langerhans Islets Vitally Stained by Intravenous Injection of Dithizone and Azo-Compounds of Quinoline Family

Reagent		Color of reagent-zinc compound	Experiments with rabbits (2050～3000 g. body weight)							Experiments with mice (15～23g. body weight)						
			Dose	Color of islets vitally stained	Degree of vital staining after the administration of reagents					Dose	Color of islets vitally stained	Degree of vital staining after the administration of reagents				
Name	Color when dissolved		(mg./kg., intravenously)		ca.5 min.	ca.10 min.	ca.20 ～30 min.	ca.1 ～2 hrs.	ca.3 ～4 hrs.	(mg/kg, intravenously)		5 min.	10 min.	20 ～ 30 min.	1 hr.	3 hrs.
Dithizone	Red	Purple red	100～200	Purple red	++	+++	++++	+++	+	100～200	Purple red	++	+++	++++	+++	+
5-(Phenylazo)-8-hydroxyquinoline	Carmine	Orange-yellow	25	Orange-yellow				++		25～100	Orange-yellow	++	++	+++	++	±～−
5-(Phenylazo)-8-hydroxyquinaldine	Red	〃	10, 20	〃			+	−		20～ 50	〃	±	+	+		−
5-(*p*-Tolylazo)-8-hydroxyquinoline	〃	〃								60		−		−		−
5-(*o*-Tolylazo)-8-hydroxyquinoline	Carmine	Orange								60				−		−
5-(*p*-Hydroxyphenylazo)-8-hydroxyquinoline	Dark carmine	〃	50, 40	Orange	+++		++++	+++		100～200	Orange	++	+++	++++	++	±～−
5-(*m*-Hydroxyphenylazo)-8-hydroxyquinoline	〃	〃								70～150	〃	++	+++	+++		±～−
5-(*p*-Hydroxyphenylazo)-8-hydroxyquinaldine	Red	〃								80～140	〃	+	++	++	+	±～−
5-(*p*-Dimethylaminophenylazo)-8-hydroxyquinoline	Dark carmine	Orange-yellow	60, 50	Orange-yellow	++		+++			90	Orange-yellow	++		+++		±～～
5-(*p*-Dimethylaminophenylazo)-8-hydroxyquinaldine	〃	〃								50		−		−		−
5-(*p*-Acetaminophenylazo)-8-hydroxyquinoline	〃	Orange								80～120	Orange	+	++	++++		±～−
5-(*p*-Acetaminophenylazo)-8-hydroxyquinaldine	Red	Orange-yellow (light)	10	Orange-yellow (light)			+			40～ 50	Orange-yellow (light)	+	++	++～+	±	−
5-(*p*-Aminophenylazo)-8-hydroxyquinoline	〃	〃	20	〃			+			80～120	〃		+	++		−
5-(*p*-Aminophenylazo)-8-hydroxyquinaldine	〃	〃								80～100	〃		+	++～+		−

bonate are β-cell-injuring but not diabetogenic.

That these diabetogenic reagents readily combine into compounds with zinc and that as cited above, all the hitherto known diabetogenic reagents, such as alloxan, dithizone, oxine, as well as potassium ethylxanthate reported by Kadota and Midorikawa (1951)[35] (with low diabetogenic efficacy) and dehydroascorbic acid reported by Patterson[36] are all alike in this respect[37]; that no substance that is not zinc-affine, even if affine with metals with similar properties as zinc, such as copper, cadmium, cobalt and nickel, has been found diabetogenic to date; that even in the case of compounds very similar to diabetogenic substances in structural formula, these cease to show β-cell injuring and diabetogenic effects once they lose their zinc-affinity (for example, of the duos of alloxan and dibromobarbituric acid, oxine and 8-methoxyquinoline, 8-hydroxyquinaldine and 4-hydroxy-8-methoxyquinaldine or 4,8-dihydroxyquinaldine, 2,4--dimethyl-8-hydroxyquinoline and 4,8-dihydroxyquinaldine, diabetogenic quinoline-azo-derivatives and 5-(p-nitrophenylazo)-8-hydroxyquinoline or 5-(p-nitrophenylazo)-8-hydroxyquinaldine, the first named are zinc-affine and diabetogenic while the second are not); that colored diabetogenetics can be observed to come into combination with the cells of Langerhans islets with preference morphologically, and the color of the vital staining by them is exactly identical with the color of the compound of zinc and the reagent, so that it has been evidenced that these reagents directly combine with β-cells upon administration, leading to lesions in them and to diabetization, are all findings in perfect consonance with and in support of Okamoto's zinc theory of diabetes. A considerable number of zinc affine substances, however, have been found to induce no diabetes or to cause no severe lesions to β-cells, but there is an explanation to offer in almost all cases of such seeming exceptions, as given below and it is of course inappropriate to doubt the zinc theory on this score.

(1) Among zinc-affine compounds, there is a group of compounds having no β-cells toxicity or diabetogenic efficacy either (a) similar to or (b) dissimilar to diabetogenic reagents in their structural formulae, for example, (a): 5-nitrobarbituric acid and sodium rhodizonate (similar in structure to alloxan), xanthurenic acid, quinaldinic acid and atophane (resembling diabetogenic quinoline derivatives), (b): Benzimidazole, benzotriazole, bismuthiol I, morin, salicylaldoxime, anthranilic acid, cholic acid, theobromine, cystine, norleucine, creatinine. These compounds are effective in preventing occurrence of alloxan diabetes, as will be separately reported (and have been reported in part).[6,12] These do combine with Langerhans islet cells, quite as the diabetogenic substances, but being not or only weakly toxic to β-cells do no injure them, but blockade them against the subsequently introduced alloxan, so that it is prevented from combining and diabetes is not produced.

(2) When SO_3H, COOH or CHO bases are affixed to the diabetogenic quinoline compounds and the phenyl bases of quinoline-phenyl-azo compounds, the zinc affinity of these compounds are not lost, yet they become non-diabetogenic. This is seemingly due mainly to their loss of cytotoxicity and also to their facility in being excreted.

(3) 5-(*p*-tolylazo)-8-hydroxyquinaldine°, anabasin°, brucin°, potassium or lithium urate°×, 5-bromoanthranilic acid×, thionalide°, rubeanic acid°, *o*-phenanthrolin hydrochloricum°, 5-nitroquinaldinic acid×, 8-nitroquinaldinic acid× and indoxine° are also zinc-affine but do not induce diabetes. Here, we may understand that these marked with ° are not sensitive enough in their reaction with zinc and cannot be administered in doses large enough to make effectively extensive combination in Langerhans islets or that the × marked ones are almost non-toxic against cells.

(4) The compounds with Br, NO_2, NO or $AsO(OH)_2$ subjoined to the phenyl bases of the phenylazo derivatives of oxine and 8-hydroxyquinaldine, 4-hydroxybenzthiazole, 2-benzothiazole, *o*-phenylenbiguanide, and chloranilic acid are zinc-affine indeed but show no β-cell toxicity and no diabetogenic effect. The reason is not clear, but it must be noted that, as we have pointed out in the introduction, many conditions must be fulfilled upon introduction of these reagents into the blood before they can reach the Langerhans islets and combine with zinc therein, and we may infer that some of these prerequisite conditions are not adequately met in these cases, so that the expected combination and consequently the production of diabetes are inhibited.

Next, we will examine the changes in blood sugar content appearing in the earlier stages of diabetization. Seeing that a very peculiar fluctuation of blood sugar content is observable at the incipient stage of alloxan diabetes, many studies and discussions have been conducted on the subject, but no reconciliation of opinions has yet been reached on the matter of the primary high and the subsequent low blood sugar content. As to the occurrence of the primary transient high glycemia, there have been attempts to explain its mechanism by an increase of glycolysis by an action of alloxan exerted indirectly upon the liver through the tarnsmission by the adrenaline-sympathicus system,[23,38,39,40,41] by increase of glycolysis by direct action of alloxan on the liver,[42,43] by suspension of insulin secretion,[3,6] by the action of alloxan upon the hypophysis or the adrenal cortex, or the hypophysis-adrenal cortex system[44,45,46,47] and by excitation of the α-cells,[48] but in our experiments reported herein we could obtain no results adequate in clearing up the problem.

The subsequent abnormal drop of blood sugar content is generally attributed to a temporary release of a large quantity of insulin,[24,25] but Houssay *et al.*[43] assert that this is due to the stoppage of glucose supply from the liver following the release of carbohydrates from the liver in the antecedent period of heightened blood sugar content. Okamoto[6,8] has previously expressed his opinion in favor of the generally accepted insulin release theory, based upon his results obtained from his experiments in which had administered a quantity of alloxan and dithizone too small for inducing diabetes and cause lesions in β-cells to an extent and several days afterwards, given the required dosis to effect diabetization, and had found that the primary high glycemia directly passed over into the final permanent glycemic state without passing through a state of low blood sugar content. In the experiments reported herein, we have arrived at results corroborating this opinion, for we found that (1) as in the case with administration

of 5-(*p*-hydroxyphenylazo)-8-hydroxyquinoline, 5-(*p*-dimethylaminophenylazo)--8-hydroxyquinoline and 5-(*p*-acetaminophenylazo)-8-hydroxyquinoline the stage of low sugar content is entered upon without the preceding primary high glycemia, (2) the stage of low blood sugar content begins at different times after the injection according to the variety of the diabetogenic reagent, ranging from 1.5 to 12 hours, the difference between the early and the late appearing times being well defined and this timing is in agreement with that of histological changes of necrosis and necrobiosis of the β-cells, and (3) some reagent, such as 5-(*p*-acetaminophenylazo)-8-hydroxyquinaldine, 5-(*p*-aminophenylazo)-8-hydroxyquinoline and 5-(*p*-aminophenylazo)-8-hydroxyquinaldine cause only inperceptible drop of blood sugar content following an exceptionally high primary glycemia, and we are led to the inevitable conclusion that the abnormal drop of blood sugar content in the earlier stage of diabetes artificially induced is caused by a temporary but active release of insulin by the β-cells.

Summary

1. Acting upon the idea on occurrence of diabetes published previously by Okamoto, we conducted our studies with 87 sorts of chemicals selected mainly from zinc-affine reagents and also from substances similar to these in structural formulae and closely related chemically to these and have arrived at the following results:

(1) We have discovered 17 new substances effective in producing diabetes in rabbits, namely, 8-hydroxyquinaldine, 2, 4-dimethyl-8-hydroxyquinoline, 13 substances with phenylazo bases (phenylazo, tolylazo, hydroxyphenylazo, dimethylaminophenylazo, acetaminophenylazo and aminophenylazo bases) in the position 5 of oxine and 8-hydroxyquinaldine, diphenylthiocarbazide (which, however, apparently changed into dithizone while preparing the injection solution) and nicotine. Except nicotine, all these compounds being insoluble in water, were used dissolved in alkaline solvents or hydrochloric acid under room temperature or by applying heat.

(2) The minimum dosis required for inducing diabetes has been estimated to range from 5 mg./kg. in the smallest to 45 mg./kg. in the highest for quinoline compounds, from several mg./kg. to 10 mg./kg. for nicotine and for diphenylthiocarbazide 10~20 cc./kg. of saturated solution in saturated aqueous solution of lithium carbonate.

(3) These substances injured the β-cells of Langerhans islets and induced permanent diabetes by one single intravenous injections except nicotine which gradually cause severe lesions to β-cells upon 3 or 4 administrations at 5~15 days' intervals in doses of several mg./kg. and finally induces diabetes. In the cases of severe diabetization, the β-cells of the Langerhans islets were reduced to below 12 (below $\frac{1}{3}$ of the normal), and in most cases to below 8 (below $\frac{1}{5}$ of the normal) in number per islet.

(4) At the earliest stage of administration of a reagent, excepting nicotine, a marked fluctuation was seen in blood sugar content. There are 3 major

types of this fluctuation. (a) In the majority of cases, the blood sugar content temporarily rose at first (about 3,6 or 21 hours after administration), then dropped widely (about 3～10, 6～12 or 12～24 hours after administration), rose to permanent high glycemia thereafter and passed over into diabetes. Most of the reagents cause such a type of fluctuation of blood sugar content. (b) Some reagents do not cause the temporary heightening of blood sugar content, but a strong fall of it in 1～2 hours after the administration and then the permanent high glycemia soon sets in. Diabetes is induced in a short time in these cases. 5-(*p*-hydroxyphenylazo)-8-hydroxyquinoline, 5-(*p*-dimethylaminophenylazo)-8--hydroxyquinoline and 5-(*p*-acetaminophenylazo)-8-hydroxyquinoline belong to this type of reagents. (c) The three phases of fluctuation is indeed complete following administration of a few other reagents, but the stage of low blood sugar content is not so distinct here.

When the drop of blood sugar content was marked, the following diabetes was also severe. The rise and fall of blood sugar content runs abreast with the lesion of β-cells, the time of abnormal drop of blood sugar content just coinciding with the time of widest necrosis of β-cells. This seems to prove that this drop of blood sugar content is attributable to a sudden release of insulin.

(5) 5-methyl-8-hydroxyquinoline, 8-hydroxy-2-(α-methyl)quinoline-methanole, 4-methylthiouracil, diphenylcarbazide, sodium diethyldithiocarbamate and guanidine carbonate are toxic against the β-cells but the toxicity does not reach threshold of diabetization. That is, the reduction of the number of β--cells due to the injury is limited to the extent of dropping to $\frac{2}{3}\sim\frac{1}{3}$ of the normal count and this is not sufficient to cause diabetes.

(6) The diabetogenic reagents are all zinc-affine in nature and even substances very similar to these substances in structural formulae or closely related with them are not effective in causing diabetes, once their zinc-affinity is lost.

(7) We found a considerable number of zinc-affine substances that cause no lesion in β-cells and are thus not diabetogenic. Most of these were not or only weakly cytotoxic (these act prophylactic against diabetization), but some were understood to be ineffective because only a too small dosis could be used or were too readily excreted.

2. Upon observing the progress of diabetes induced in rabbits with 16 of the newly discovered diabetogenic reagents and the known diabetogenic reagents alloxan, dithizone and oxine, we obtained the following results:

(1) Many of the cases made diabetic with oxine, 5-(*p*-acetaminophenylazo)--8-hydroxyquinaldine, 5-(*p*-aminophenylazo)-8-hydroxyquinoline, 5-(*p*-aminophenylazo)-8-hydroxyquinaldine and nicotine improved and recovered in the course of observation. This is seemingly due to the lightness of the lesions in the β-cells, leading to a speedy regeneration of them.

(2) When other reagents than the above 5 were administered, the induced diabetes was generally severe. Of the 121 cases tested, about 50% had their illness persisting for more than 100 days, about 20% for more than one year and 3.3% for more than 2 years, the rest either recovering or dying in the respective periods. The ratio of fatal and cured cases always stood at 3 :1 on the 100th

day, 1 year, 2 years, and 3 years after the diabetization. The longest case of continuous diabetes persisted for 1,100 days and the next longest for 850 days.

(3) The leading cause of death among the diabetic rabbits was diarrhea, taking a toll of 35% in all stages of the progress, followed by 22% of strong lipemia at the time of death, most frequent in the first 20 days of diabetization, by 15% of suppurative diseases oftenest coming on later than 2 months after diabetization, and by 14% of death from marasm and bad appetite, the former most frequent more than 100 days after and the latter in the first stage of diabetization. No one died of cardio-renal-vascular diseases, the leading cause of death among human diabetes patients.

3. The 5-phenylazo compounds of oxine and 8-hydroxyquinaldine are all pigments, and a microscopic examination of fresh Langerhans islets taken from rabbits and mice intravenously injected with them revealed to following:

(1) With 10 of the 13 reagents used, the islets showed positive vital staining, as with dithizone and with 5-phenylazo-8-hydroxyquinoline, 5-(*p*-hydroxyphenylazo)-8-hydroxyquinoline, 5-(*m*-hydroxyphenylazo)-8-hydroxyquinoline and 5-(*p*-acetaminophenylazo)-8-hydroxyquinoline it was especially bright. No other cells or organs outside the islets were stained.

(2) Though the solutions of the reagents are red, carmine or dark carmine in color, the vital staining of the Langerhans islets appeared in the color of orange-yellow or orange, color tones identical with these of the zinc compounds of the reagents. This shows that the vital staining of the islets occasioned by these reagents is a result of chemical combination of zinc in the islets with the reagents, as it was the case with dithizone.

(3) The vital staining of the islets is clearly perceptible 5 minutes after the administration already, reaching the maximum density in 20～30 minutes, then gradually fading off, to loss visibility in 3 hours. Thus, we may conclude that the diabetogenic reagents directly combine with zinc in the islets upon administration and then bigin to cause lesions in them.

4. Considering the findings obtained to date in collation, we are led to deem the zinc theory by Okamoto as most universally applicable concerning the mechanism of diabetes production with any diabetogenic reagents, including alloxan, dithizone, oxine and various quinoline compounds.

II. Studies on Prevention of Experimental Diabetes Mellitus by Means of Some Chemical Substances*

Since the discovery of alloxan diabetes,[3,4] there have been many studies reported on diabetes mellitus with alloxan used in the experiments while the prevention side of this disease has not at all been neglected.[34,49~62] The authors have also undertaken a research on the prevention of alloxan diabetes in general, and in particular, on the prevention of dithizone diabetes[6] and the quinoline derivatives diabetes[6,11] discovered by Okamoto and his collaborators. This research of ours had two outstanding objects among the others. In the first place, we aimed at obtaining reference data illuminating the preventive methods of diabetes, and secondly, we hoped to elucidate the mechanism whereby these chemicals induced diabetes. Some considerations relevant to these two points will be discussed in the following.

Many substances have been reported to show diabetogenic effect, such as alloxan, dehydroascorbic acid,[36] dithizone, oxine,[6] nicotine[8] and quinoline derivatives.[11] Dehydroascorbic acid exists in our organism physiologically, though, maybe, in a small quantity,[36] while alloxan and oxine might be produced therein—the former from uric acid or vitamin B_2[24,25] and the latter from tryptophane.[63] Nicotine is introduced into our system from tobacco. Thus, it may be seen that these substances may possibly cause diabetes in us, and it follows that the research for the prevention of diabetes produced by these substances may very probably result in the discovery of a preventive method of diabetes from which we humans are suffering.

Now, let us follow the destiny of a diabetogenic reagent introduced into our system. It is first subjected to detoxication and decomposition in the blood and some organs, to be finally ejected out of the body through the excretory organs. However, a part of it goes into the pancreas and attacks the β-cells of the Langerhans islets. Thus it follows that for the prevention of diabetes in this case, either (1) the detoxicative, the decomposing and the excreting functions must be fortified, or (2) the efficacy of the reagent in injuring the Langerhans islet cells must be weakened, or again (3) the defensive and regenerative abilities of the said cells must be fostered. The method (2) above, as it consists, so to speak, in the control of the diabetogenic mechanism itself, can be studied only in close relation with the elucidation of the said mechanism.

It is regrettable, however, that the opinions of the researchers are divided in regard to the diabetogenic mechanism. Lazarow[34,55] has published his glutathione theory, while Okamoto[6] has his own zinc theory of diabetes. The former stands on the assumption that the β-cells in the Langerhans islets are poorer in glutathione in comparison with cells in other tissues. Consequently, when the

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glutathione content in the body is severely reduced by alloxan administration, the β-cells are depleted at the very first, so that their alloxan tolerance is undermined and they fall prey to its injurious effect, leading to the production of a diabetic state. According to this theory, it would be possible to prevent diabetes by a large dosis of glutathione, or, since the effective factor in glutathione in fighting alloxan is known to be the SH radical in it, it would follow that administration of a substance containing SH radical would be equally effective in preventing alloxan diabetes. In fact, studies have been conducted on a variety of reagents containing SH radicals or reagents effective in creating SH radicals in animal organisms, as presumably effective in preventing alloxan diabetes.[51~55]

As stated in the preceding chapter, Okamoto propounded his Zinc Theory of Diabetes Mellitus in 1949. The theory implies that zinc-affine substances injurious to tissue cells react diabetogenically, while such substances only weakly or non-injurious to tissue cells react diabetopreventively. In particulars, a non-injurious (or slightly injurious) zinc-affine substance will combine with the zinc in the β-cells and exclude the cell injurious diabetogenic zinc-affine substances from combining therewith. According to this theory, the non-injurious, or the slightly injurious, zinc-affine substances must be effective in preventing experimental diabetes mellitus. It follows that the results of the tests for which zinc-affine substances, beside the SH radical containing reagents and the substances promoting production of SH radicals in animal systems, are effective in the prevention of diabetes, and how the mechanism of prevention works—whether by prevention of the reduction of the glutathione content or by blocking of the β-cells against further combination of its zinc by a prophylactic combination of its zinc with benign zinc-affine substances—will serve as decisive data in a comparative criticism of these two theories—the Lazarow's glutathione theory and Okamoto's zinc theory.

Experimental

Materials and Method

Substances used for the experiment of prevention: From the infinity of chemicals available for our experiments, we selected 86 varieties, with the four following principles as our standards: (a) According to Okamoto's zinc theory, 33 non- or slightly cell injurious zinc-affine reagents were chosen (a part of our experiments with anthranilic and quinaldinic acid has been reported previously).[6,8] (b) Acting upon our experiences that a diet rich in vitamin B induced difficulty in diabetogenesis by alloxan and similar drugs, and the reported observation[63,64] that a deficiency in vaitmine B_1 and B_6 causes or tends to cause production of diabetogenic oxine or the closely related quinoline derivatives from tryptophane and their production is stopped by administration of the deficient vitamines, we tested 14 varieties of vitamins. (c) We reexamined the efficacy of 16 reagents reported as effective or not in the past.[24,49,52,54,56,59,60] (b) Substances resembling in their constitution with the reagents included in the three categories above and proved effective in our experiments, 38 in total varieties, were also tested.

(The total of the number cited in (a)～(d) above foots up to 101, but 15 of the items come under two headings at once, so the actual number of items is 86).

The test animals: These consisted of male white rats more than 100 g. each in body weight, male rabbits of more than 2 kg. each and guinea pigs of more than 300 g. each. For ten days prior to the experiments, fodders rich in vitamin B were avoided. Following the administration of the tested reagents, the white rats were fed with rice, carrots, potato and other vegetables and small fish, and the rabbits and guinea pigs with bean curdle refuse, oats, carrot, sweet potato, weeds and other vegetables. Sufficient water to drink was given them.

Diabetogenic reagents: For these, alloxan made by Eastman Kodak Co., dithizone made by Wakō Junyaku Kōgyō K.K. and 8-hydroxyquinaldine, synthesized by Asst. Prof. Matsuo of Kōbe Women's College of Pharmacy and oxine made by Takeda Seiyaku K.K. were used. Alloxan was used in 5% aqueous solution, dithizone[6] in a saturated solution in 0.3% aqueous ammonia, prepared by dissolving 100 mg. of dithizone in 10 cc. of ammonia solution kept at 70°C for ten min., and 8-hydroxyquinaldine[11] and oxine[6] were used in 0.5% solution made by titrating dilute hydrochloric acid over them to dissolve and then further diluted down with water. These solutions were prepared just before the use in every case.

Experimental methods and appraisal of results: (a) Experiment on prevention of alloxan diabetes in white rats: The tested reagents were used in water solution wherever possible, but the water insoluble chemicals were dissolved in NaOH solution, alcohol or HCl solution as the occasion demanded, and given intravenously to the animals 5 min. before the administration of alloxan. Substances not adapted to venous injection were administered subcutaneously or intramuscularly 30 min. prior to the alloxan injection. 50 mg./kg. of alloxan was then injected into the femoral vein in 5% aqueous solution. The animals were kept under observation for 2～10 days after the operation and then sacrificed for autopsy. Throughout the period of observation, the urine sugar was tested daily by Benedict's reagent (please refer to Chapter I). Negative urine sugar at every test 24 hours after the operation and downwards, normal blood sugar content at the time of sacrificing as measured by Hagedorn-Jensen's method (150 mg./dl. or less), and absence of noticeable damage to the tissue of the Langerhans islets upon microscopic study of pancreatic tissue sections stained with Gomori's method,[19] especially retention of more than 20 β-cells* per islet, were deemed as indicative of satisfactory preventive effect against experimental diabetes.

Then the administrated doses of the tested reagents were changed according to a fixed schedule of scales and the minimum effective dose for prevention was determined for each reagent. In the case when with the maximum dose allowable for administration (an amount approaching the fatal dose) the reagent proved ineffective in prevention, that is, if the blood sugar content was over 180 mg./dl., the urine sugar showed +～卌 or the β-cells fell below 20 per islet, the reagent was declared ineffective as a prophylactic.

The above minimum effective doses of the reagents were determined under

the condition of their injection 5 min. before the administration of the diabetogenics, when intravenous, and 30 min. before when given subcutaneously or intramuscularly. Then, the doses were fixed and the time interval was changed (immediately, 1, 3, 5, 30 minutes, 1, 3 and 6 hours), to determine the effective period of prevention.

*: Estimation of the number of β-cells in Langerhans islets and its significance: The number of cells in the islets was counted as described in Chapter I.

In our experiments with white rats made diabetic by administration of alloxan of 30, 40 and 50 mg. per kg. of body weight, we found that 18 β-cells per islet was the maximum value to be encountered in diabetic pancreas, and when the value was not less than 20, no diabetes could be diagnosed symptomatically (urine sugar − and blood sugar less than 150 mg./dl.), from our examination of 5 non-diabetic cases, 6 mild cases and 5 healed cases after a passing attack.

(b) Experiments on prevention of alloxan, dithizone and 8-hydroxyquinaldine diabetes in rabbits: Promising reagents among those found effective in preventing alloxan diabetes in white rats were used in similar experiments with rabbits. Solutions of the tested chemicals prepared in a similar manner as those used in the experiments with the rats above, were injected into the ear veins 5 min. before the administration of the diabetogenic substances. Substances unadapted for venous injection were injected under the dorsal skin 30 min. prior to the injection of the diabetogenics. The diabetogenics were used in the following doses: alloxan, 120 mg./kg.; dithizone, 10 cc./kg.; 8--hydroxyquinaldine, 30 mg./kg.. After the operation, blood sugar was measured by Hagedorn-Jensen's method and the urine sugar by Benedict's reagent, at the elapse of 3, 6, 9, 12, 18 and 24 hours, and once every day thereafter. The methods employed in the estimation were the same as that used with the white rats.

(i) If at the end of hours the blood sugar has not risen above 150 mg./dl.; (ii) the urine sugar was found negative and also (iii) the β-cells numbered not less than 11 per islet (a number found to represent the maximum in diabetic pancreas of rabbits based on observations at this laboratory),[8] the tested reagent was decided as effective, and when only the result (iii) was apparent, without being supported by (i) and (ii), the reagent was labeled as partially effective.

(c) Experiments on prevention of oxine diabetes in guinea pigs: Some of the reagents declared effective in the experiments with the alloxan diabetic white rats were used in determining the preventive effect against oxine diabetes induced in guinea pigs. The tested reagents were prepared in the same manner as with the white rats and were injected into the femoral veins or beneath the dorsal skin, 5 min. or 30 min. after the last injection, oxine was administered through the femoral vein at the ratio of 120 mg. per kg. of body weight. After three days' observation, they were killed. The methods of measuring the urine sugar and the blood sugar content and the histological examination were the same as described above as applied to white rats. When the blood sugar was found to be below 150 mg./dl., the urine sugar negative and the number of β-cells in the Langerhans islets decidedly above the number in the control which was subjected to oxine injection only, the reagent was considered effective.

Experiments on the elucidation of the mechanism of prevention of diabetes by specific preventive substances and their evaluation:

(a) Mechanism of prevention

As has been described in the Introduction, the mechanism of prevention of diabetes induced by diabetogenic reagents can be attributed to one of the three following causes: (1) The diabetogenic substance is disposed of by detoxication or decomposition by blood or some organ in the body or by the action of a preventive substance; (2) The diabetogenic substance is prevented from attacking the β-cells; or (3) The β-cells themselves acquire greater resistance against the diabetogenics. In cases (2) and (3), the prevention was realized inside the Langerhans islets, while in case (1), it has been brought about outside the islets. We tried to clarify the mechanism of the prevention but our efforts were not rewarded as we hoped for, though at least we could substantiate whether the action was carried out within or without the pancreas.

(b) Preliminary experiment to interpret the mechanism of prevention

Now, cut open the abdominal wall of a white rat and clamp the pancreas between a pair of forceps at the spleen side, that is, grip the large blood vessel running to the spleen side of the pancreas forcibly (see Fig. 35 (A)), so that the circulation to the cauda pancreatis or the spleen side is held up, and you will see the blood circulation in all the blood vessels in the pancreas at once reflexly suspended. This interruption of circulation can be kept up for at least 30 min. almost without causing any derangement in the circulation to the other organs. This fact has been confirmed by our experiment with 20 white rats as objective, in which, immediately upon suspending the local circulation as described above, we injected pigments, such as indigocarmine, sodium rhodizonate or dithizone in respective doses of 10 cc./kg. of 1% aqueous solution, of 30 cc./kg. of 0.5% aqueous solution and of 20 cc./kg. of saturated solution at 70°C in 0.3% aqua ammonia into their blood, and every 5 minutes examined their organs including the pancreas macroscopically and microscopically. This means, that it is practically possible to suspend selectively the blood circulation to the pancreas by such an extremely simple means, for a required length of time, at least for 30 min. in succession.

(c) Methods of experiments for interpreting the mechanism of prophylaxis and of estimating the results

The experiments for interpreting the mechanism of the prophylaxis were conducted as based on the practical application of the experimental results in (b) above, consisting of the two processes as follows:

1) Experimental methods (Fig. 35 (B))

(1) The first method: As described in (b) above, the pancreas was kept pressed between a pair of forceps to stop blood from circulating therein, the effective dosis of prophylactic reagent was applied and the forceps were detached 5 minutes thereafter (30 minutes after, in the case when the reagent was applied intramuscularly or subcutaneously), and directly upon the resumption of normal circulation (about 10~15 seconds after detaching the forceps), 50 mg./kg. of alloxan was intravenously injected. In other words, this method aims at the

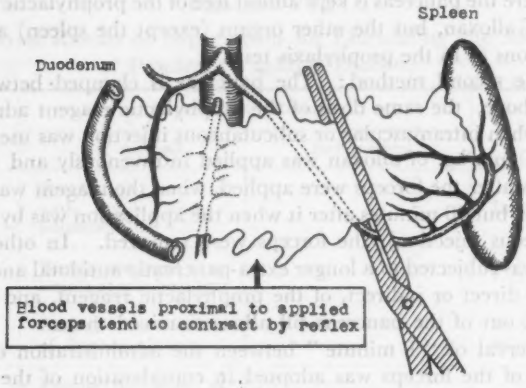
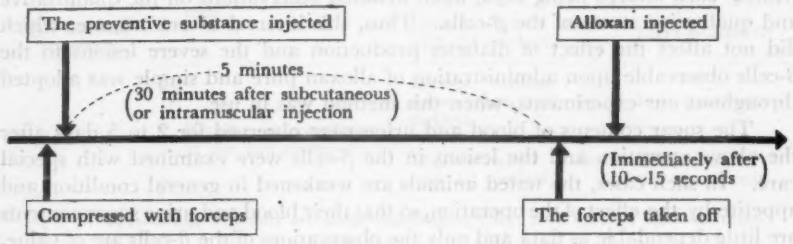
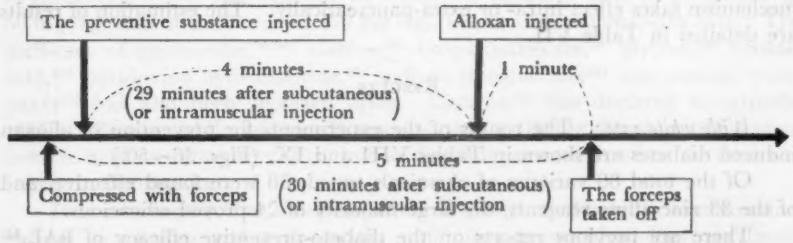


Fig. 35 (A): Explanatory diagram on the mechanism of preventing experimental diabetes.

Method 1:



Method 2:



(B): Experimental methods for the mechanism of preventing diabetes.

situation where the pancreas is kept almost free of the prophylactic reagent before the attack of alloxan, but the other organs (except the spleen) are kept in the same conditions as in the prophylaxis tests.

(2) The second method: The pancreas is clamped between a pair of forceps, as above, the same dosis of the prophylactic reagent administered and 4 minutes (when intramuscular or subcutaneous injection was used, 29 minutes) thereafter, 50 mg./kg. of alloxan was applied intravenously and 1 minute later (or 5 minutes after the forceps were applied, when the reagent was administered intravenously, but 30 minutes after it when the application was by intramuscular or subcutaneous injection), the forceps were removed. In other words, here the alloxan was subjected to a longer extra-pancreatic antidotal and decomposing effect, either direct or indirect, of the prophylactic reagent, and the latter was severely kept out of the pancreas till alloxan arrived there.

This interval of "1 minute" between the administration of alloxan and the removal of the forceps was adopted in consideration of the results of our experiments with white rats, wherein we found that if the forceps affixed to the pancreas are withdrawn within 2 minutes after the application of alloxan, and better within 30 to 60 seconds, the following findings on the lesions brought upon Langerhans islets was entirely similar to those following alloxan administration without such forceps being used, upon detailed observations on the quantitative and qualitative status of the β-cells. Thus, this interval of one minute, which did not affect the effect of diabetes production and the severe lesions to the β-cells observable upon administration of alloxan pure and simple was adopted throughout our experiments, when this method was in use.

The sugar contents of blood and urine were observed for 2 to 5 days after the above operation and the lesions in the β-cells were examined with special care. In such cases, the tested animals are weakened in general condition and appetite by the effect of the operation, so that their blood and urine sugar contents are little dependable as data and only the observations of the β-cells are of value.

2) Inference of the mechanism of prophylaxis from the experimental results

By applying the 1st method stated above only or both the 1st and the 2nd methods in combination, it is possible to determine whether the prevention mechanism takes effect intra- or extra-pancreatically. The estimation of results are detailed in Table VII.

Results

With white rats: The results of the experiments for prevention of alloxan induced diabetes are shown in Tables VIII and IX. (Figs. 36~50.)

Of the total 86 varieties of chemicals tested, 38 were found effective, and of the 33 zinc-affine reagents, the large majority of 24 proved efficacious.

There are previous reports on the diabeto-preventive efficacy of BAL,[54] nicotinamide,[56] glutathione,[34] cysteine[34,52] and atophane,[59] as well as of sodium bisulfite[49] similar to sodium thiosulphate in constitution. Of sodium thiosulphate, Weinglass *et al.*[49] have declared the dosis of 680 mg./kg. as ineffective, upon experimenting on alloxan diabetes in rabbits and also sodium bicarbonate

TABLE VII

Estimation of Results of Experiments for Elucidating the Mechanism of Prophylaxis against Diabetes

	Experimental Results		Decision
	1st method	2nd method	
Slow-acting reagents*	Results the same as with simple alloxan administration		Intra-pancreatic
	Results the same as in simple prophylaxis tests		Extra-pancreatic
	Results intermediate of the two above		Intra- and extra-pancreatic
Quick-acting reagents†	Results the same as with simple alloxan administration		Intra-pancreatic
	Results the same as in simple prophylaxis tests	Lesions similar or severer than with 1st method	Intra-pancreatic
		Lesions lighter than with 1st method	Extra-pancreatic
	Results intermediate of the two above	Lesions similar or severer than with 1st method	Intra-pancreatic
		Lesions lighter than with 1st method	Intra- and extra-pancreatic

* Slow-acting reagents are those manifesting effect 2～3 minutes after administration or later. † Quick-acting reagents are those that show effect against alloxan injected immediately following their administration.

as ineffective at 500 mg./kg., but we found the effective dosis of the former as 50 mg./kg. and of the latter as 100 mg./kg., in our experiments with rats. The inefficacy of methionine,[52,54] alanine,[34] Ca-pantothenate,[59] glycine,[59] benzoic acid,[59] pyridoxine hydrochloride,[59] sodium thioglycolate[52] and sodium phosphate[49] has also been reported upon. Lazarow[34] has declared thioglycolic acid as effective, while Banerjee[59] reported that a 100 mg./kg. dose of *p*-aminobenzoic acid was non-preventive, but we found it preventive, when the dose was raised to 500 mg./kg..

It is noteworthy that vitamins of the B group, cholic acids, theobromine, sodium bicarbonate and amino-acids, so familiar to our physiology, are found among the diabeto-preventive substances. It goes without saying that substances efficacious in smaller dosis and for a longer period should be deemed superior as prophylactics and from such a viewpoint, it is highly instructive that vitamin B, particularly B_{12} takes the lead, followed closely by cholic acids that

TABLE

Results of Successful Experiments on Prevention

Reagent		Dose (mg./kg.)	Minimum effective dose (mg./kg.)
Name	Solution		
Normal (6 cases)			
Control (10 cases)			
Benzimidazole*	0.5% in 7% alcohol	5, 10, 25, 50, 100	25
Benzotriazole*	1% in aq. dest.	50, 100, 200	100
5-Nitrobarbituric acid*	2% in aq. dest.	25, 50, 100, 150, 200, 300	100
Salicylaldoxime*	1% in 5% alcohol	20, 40, 60	40
Sodium rhodizonate*	0.5% in aq. dest.	50, 100, 200, 300	200
Quinaldinic acid*	2% in 0.4% NaOH	100, 150, 300	300
Morin*	0.5% in 0.16% NaOH	50, 100, 200	200
Atophane*	2% in 0.4% NaOH	〃	200
BAL§*	10% in oil	30, 60, 120	60
Bismuthiol I*	0.5% in 0.4% NaOH	50, 100, 200	200
Sodium thiosulphate	1% in aq. dest.	10, 25, 50, 100, 200	50
Sodium bicarbonate*	2% in aq. dest.	50, 100, 200, 500	100
Borax	〃	50, 100, 250, 500, 1000	250
Vitamin B_1†	〃	50, 100, 200, 300, 400	400
Vitamin B_6† (Aderoxin, natural)	50mg. in 1cc.	100, 200, 300, 500, 1200	300
Vitamin B_{12} (Fresmin)	15γ in 1cc.	0.35, 0.5, 0.75, 0.9, 1.0	0.75
Folic acid	1% in 0.2% NaOH	100, 150, 200, 250	250
Nicotinamide	2% in aq. dest.	30, 50, 100, 150	50

VIII

of Alloxan Diabetes in Male Rats

Effective period with minimum effective dose	Effective period with twice the effective dose	Results with minimum effective dose and maximum effective period			
		Urine sugar	Blood sugar at the end of experiment (mg./dl.)	No. of cells in islet of Langerhans	
				α-cells	β-cells
		−	98±16	18.5 (16.4～19.7)	70.0 (60.0～78.1)
		|||	466 (303～510)	24.7 (18.9～29.4)	16.6 (10.9～17.5)
3 min. → 5 min.	imm. → 3 hrs.	−～±	103	30.0	34.9
		−～±	112	26.5	30.7
imm. → 1 hr.		−	102	18.0	26.1
		−～±	130	18.6	25.2
imm. → 5 min.	? → 5 min.	−～±	106	24.7	27.7
		−～±	93	28.0	31.0
? ～5 min.	? ～5 min.	−	90	26.1	26.0
		−～±	97	24.2	27.8
〃		−～±	124	22.4	25.0
		−	108	13.5	27.4
〃		−～±	110	27.2	25.7
		−～±	117	21.3	28.2
〃		−～±	115	22.1	24.3
		−	134	20.2	20.2
〃		−～±	87	17.6	23.1
		−～±	79	17.5	20.0
? ～30 min. → 1 hr.		−～±	128	18.4	48.2
		−	99	21.0	42.0
? ～5 min. → 30 min.		−～±	93	28.0	20.1
		−～±	112	24.2	23.3
imm. → 5 min.	? ～5 min.	−～±	108	17.0	26.1
		−～±	133	22.4	24.2
〃	? ～5 min. → 30 min.	−～±	116	23.8	29.7
		−	88	23.0	26.2
〃	? ～5 min.	−～±	82	17.5	24.2
		−	106	23.3	20.1
30 min.		−	97	19.4	31.7
		−～±	77	23.3	36.9
〃		−～±	102	20.6	20.0
		−～±	118	16.6	20.1
3 min. → 30 min.		−～±	113	21.2	30.6
		−～±	120	12.3	33.3
3 min. → 5 min.		−～±	93	18.1	37.3
		−	100	20.3	35.0
〃	? ～30 min.	−～±	98	22.6	45.2
		−～±	108	14.9	34.2

Reagent		Dose (mg./kg.)	Minimum effective dose (mg./kg.)
Name	Solution		
Normal (6 cases)			
Control (10 cases)			
Anthranilic acid*	3g. in 22cc. of 1N-NaOH	100, 150, 200, 300, 400, 500, 1000	200
p-Aminobenzoic acid	1% in 0.2% NaOH	200, 300, 400, 500, 1000	500
Thiochrome	1% in 0.1% HCl	100, 150, 200, 400	200
Hesperidine	1% in 0.2% NaOH	100, 150, 200, 300	150
Cholic acid*	0.5% in 0.2% NaOH	30, 50, 100, 150	50
Glycocholic acid*	〃	30, 50, 100	50
Desoxycholic acid*	〃	30, 50	50
Glycodesoxycholic acid*	〃	30, 50, 100	50
Hyodesoxycholic acid*	〃	50, 80, 100	100
Ursodesoxycholic acid*	〃	50, 100	100
Hippuric acid*	〃	50, 100, 200, 300, 400	100
Theobromine*	〃	50, 100, 150, 180, 200	180
Glutathione	5% in aq. dest.	300, 400, 500, 1000	500
Dopa	1% in aq. dest.	30, 70, 100, 150, 500	70
Tyrosine	2% in 0.4% NaOH	100, 300, 400, 600, 700	400
L-Cysteine	1% in aq. dest.	50, 80, 100, 500	100
L-Cystine*	2% in 0.5% NaOH	50, 100, 300	100
DL-Norleucine*	2% in 0.2% NaOH	100, 200, 400, 500, 1000	500
DL-Creatinine*	10% in aq. dest.	500, 1000	500
Xanthurenic acid*	2% in 0.5% NaOH	300, 500, 1000	1000

* Zinc-affine reagents. † Preventive substance was injected in the muscle.
§ Preventive substance was injected subcutaneously. ? No prophylaxis test was made.

Effective period with minimum effective dose	Effective period with twice the effective dose	Results with minimum effective dose and maximum effective period			
		Urine sugar	Blood sugar at the end of experiment (mg./dl.)	No. of cells in islet of Langerhans	
				α-cells	β-cells
		−	98±16	18.5 (16.4～19.7)	70.0 (60.0～78.1)
		|||	466 (303～510)	24.7 (18.9～29.4)	16.6 (10.9～17.5)
5 min.	? ～ 30 min.	−～±	131	19.2	29.0
		−～±	105	20.6	31.2
3 min. → 5 min.	? ～ 5 min.	−～±	110	21.1	27.6
		−～±	115	18.3	29.2
〃	〃	−～±	88	23.0	36.9
		−～±	115	17.8	30.5
? ～ 5 min.	? ～ 30 min.	−～±	110	23.5	34.4
		−	102	22.2	35.7
3 min. → 5 min.	? ～ 5 min.	−～±	88	28.5	24.9
		−～±	106	21.2	27.3
〃	3 min. → 5 min.	−～±	109	19.0	23.3
		−～±	101	20.2	29.1
? ～ 5 min. → 1 hr.		−～±	96	22.0	31.2
		−～±	91	19.2	29.1
? ～ 5 min.		−	95	17.8	33.2
		−～±	107	21.7	32.1
? ～ 5 min. → 30min.		−～±	104	23.2	48.0
		−	102	20.0	41.2
? ～ 5 min.		−	102	23.5	52.5
		−～±	98	19.1	44.9
3 min. → 5 min.	5 min. → 30 min.	−～±	89	19.9	24.0
		−	106	21.2	26.0
〃		−～±	101	16.6	37.6
		−	112	20.3	31.2
imm. → 5 min.	? ～ 5 min.	−～±	103	16.7	32.5
		−	97	20.1	38.7
3 min. → 5 min.	? ～ 5 min. → 1 hr.	−～±	95	30.0	69.2
		−～±	84	28.6	66.1
5 min. → 1 hr.		−～±	227	36.0	39.7
		−～±	142	17.5	39.3
"1 min" → 30 min.		−～±	115	18.4	32.5
		±	170	15.6	27.1
5 min.		−～±	141	23.8	58.0
		−～±	149	24.4	53.1
〃	? ～ 5 min. → 1 hr.	−～±	125	21.1	42.9
		−～±		26.1	41.0
? ～ 5 min. ～ ?		−～±	92	18.5	45.4
		−～±	129	21.2	43.0
〃		−～±	158	24.6	29.8
		−～±	199	27.9	22.0

" 1 min." mean that prevention was effected upon applying the prophylactic reagent 1 minute after the administration of alloxan.

TABLE IX

Results of Unsuccessful Experiments on Prevention of Alloxan Diabetes in Male Rats

Reagent		Dose	Results of maximum administered dose			
Name	Solution	(mg./kg.)	Urine sugar	Blood sugar at the end of experiment (mg./dl.)	No. of cells in islet of Langerhans: α-cells	β-cells
Theobromine sodiosalicylate*	2% in aq. dest.	250	++++ ++++	499 320	27.0 25.2	14.2 16.7
Sodium phosphate*	20% in aq. dest.	1000	++++ ++++	338 447	21.2 27.2	17.7 15.5
Triethylamine*	5% in aq. dest.	500	++++ ++++	448 398	24.0 26.8	13.2 15.2
Hexamethylenetetramine*	〃	500, 750, 1000	−∼+ ++++	155 403	19.2 19.9	20.4 14.3
Lithium carbonate*	Saturated sol. in aq. dest.	3.0 cc.	−∼+ ++++	182 983	24.2 19.0	18.3 9.8
Sodium thioglicolate	2% in aq. dest.	200	+∼++++ ++∼++++	732 314	23.8 21.2	11.2 14.3
5-Bromoanthranilic acid*	1% in 0.2% NaOH	250	++++ ++++	512 388	18.8 19.9	15.3 16.5
Guanidine carbonate*	1% in aq. dest.	200	+∼++++ ++++	314 444	26.6 24.2	17.2 15.8
2-Benzothiazolethiol*	1% in 0.5% NaOH	70	++∼++++ ++++	486 376	16.2 19.8	16.3 17.8
Vitamin B_2	Frabol, 1mg. in 1cc.	60	−∼+ +++	152 330	17.6 23.6	17.9 18.8
Pyridoxine	2% in aq. dest.	100,150,200, 300,400,500, 600,1000,1200	−∼+ ++∼++++	108 452	24.2 19.9	19.0 15.1
5-Oxyanthranilic acid	0.5% in aq. dest.	50, 100, 200, 300	+++∼++++ −∼++++	292 124	20.6 19.2	17.9 22.3
Ca-Pantothenate	2% in aq. dest.	400, 500, 600	+∼++++§ ++ §§	110 235	27.3 22.4	13.3 18.0
Biotin	0.5% in 0.2% NaOH	200	++++ ++++ §		24.0 19.3	11.5 14.9
Inosit	0.5% in aq. dest.	300	+ §§ +∼+++	135	22.5 16.3	17.7 19.5
Caffeine	1% in aq. dest.	50, 75	++++ +++∼++++	490 232	18.3 22.5	14.3 14.8
Theocin	〃	100	++++ ++++	331 285	24.3 20.1	17.1 16.2
Acetylcholine	0.05% in aq. dest.	10	++++ ++++	685 313	16.1 19.1	13.3 17.1
Pyruvic acid	2% in aq. dest.	300	++++ ++++	480 280	19.8 21.2	17.0 16.7
Lyegostine	0.05% in 10% alcohol	25 cc./kg.	+++∼++++ ++++	521 403	20.1 19.9	16.5 14.3

Reagent		Dose (mg./kg.)	Results of maximum administered dose			
Name	Solution		Urine sugar	Blood sugar at the end of experiment (mg./dl.)	No. of cells in islet of Langerhans	
					α-cells	β-cells
Cardinon	injection preparate	2.2cc.	### ###	696 441	16.8 18.2	15.7 16.4
Uric acid*	1% in 0.3% NaOH	50, 100, 300	### ##～###	331 426	23.2 18.9	17.2 17.1
Urea	5% in aq. dest.	600	### ###	256 326	21.2 20.4	16.8 15.1
Benzoic acid	1% in 0.3% NaOH	500	−～++ ##～###	127 202	22.1 22.7	19.2 18.0
Creatine	2% in aq. dest.	200, 500	### ###	437 396	29.6 27.5	17.6 15.4
L-Arginine	2% in aq. dest.	500, 1000	### ###	421 449	26.8 27.6	16.4 14.9
Arginine hydrochloride	2% in aq. dest.	500, 1000	### ###	433 389	24.8 23.9	16.1 15.3
L-Leucine	2% in 0.2% NaOH	500	##～### ###	263 396	27.3 25.8	18.4 14.9
Isoleucine	2% in aq. dest.	500	### ##～###	559 421	25.6 24.9	15.4 16.0
L-Lysine dihydrochloride	1% in aq. dest.	200, 300	### ###	385 406	23.6 26.3	18.0 16.7
DL-Threonine	2% in aq. dest.	500	### ###	464 378	24.2 25.4	17.5 15.6
Histidine hydrochloride	4% in aq. dest.	100, 200	### ###	361	26.4 24.8	16.9 14.9
DL-Citrulline	2% in aq. dest.	500	### ##～###	456 403	23.9 24.4	16.7 15.9
DL-Ornithine dihydrochloride	〃	500	### ###	655 398	27.6 25.6	15.9 15.3
Asparaginic acid	2% in 0.4% NaOH	500	### ##～###	892 409	28.6 27.7	11.8 13.9
L-Hydroxyproline	2% in aq. dest.	500	##～### ###	369	24.7 25.6	17.8 14.0
L-Methionine	〃	500	+～### ##～###	622 411	21.9 27.6	17.9 15.1
Glycine	〃	500	+～### ###	338 436	23.6 24.1	18.1 16.9
Asparagine	1.5% in 0.2% NaOH	500	+～### ##～###	675 398	26.9 23.1	15.7 14.3
DL-Phenylalanine	2% in 0.2% NaOH	500	### ##～###	468 369	24.9 26.0	17.0 15.9
α-Alanine	2% in aq. dest.	500	### ##～###	504 428	19.9 20.3	17.1 15.6
Valine	5% in aq. dest.	1000	### ##～###	458 379	24.5 19.0	16.8 15.1

Reagent		Dose	Results of maximum administered dose			
Name	Solution	(mg./kg.)	Urine sugar	Blood sugar at the end of experiment (mg./dl.)	No. of cells in islet of Langerhans	
					α-cells	β-cells
DL-Norvaline	2% in aq. dest.	500	++~+++		23.9	16.4
			++~+++	510	18.0	13.9
Glutamic acid	2% in 0.4% NaOH	500	+++	614	26.6	16.8
			++~+++	296	21.5	15.8
DL-Serine	2% in aq. dest.	500, 1000	++~+++		25.7	16.4
			+	399	21.0	13.8
DL-α-Aminobutylic acid	〃	500	+++	617	23.7	17.1
			++~+++	451	17.9	12.9
Tryptophane	2% in 0.4% NaOH	500	++~+++	197	24.8	18.6
			++~+++	366	19.0	16.9
L-Proline	2% in aq. dest.	500	++~+++	502	23.4	16.2
			++~+++	299	20.6	14.8

* Zinc-affine reagents. § Symptom of weakness (died after 2 days).

are generated in our body system.

BAL containing SH group and sodium thiosulphate, deemed equivalent to SH compounds in its reactions,[68] are strong preventive substances, but glutathione, equally provided with SH radical, is only weakly effective.

With rabbits: The results of the experiments for prevention of alloxan, dithizone and 8-hydroxyquinaldine diabetes are shown in Tables X, XI and XII. The reagents used were those deemed as representative in their efficacy in preventing alloxan diabetes in white rats.

(a) In preventing alloxan diabetes in rabbits, sodium bicarbonate, vitamin B_1 and cystine showed dependable efficacy, sodium thiosulphate, vitamin B_{12}, anthranilic acid, cholic acid and hippuric acid only a moderate effect, nicotinamide and norleucine nearly, and benzimidazole utterly, no effective.

As cited above Weinglass *et al.*[49] have asserted that sodium thiosulphate and sodium bicarbonate are ineffective in rabbits at the doses of 680 mg./kg. and 500 mg./kg. respectively, but we increased the doses to 1,000 mg./kg. and 1,500 mg./kg. respectively and found them effective in preventing diabetes to some extent.

(b) Against dithizone diabetes, sodium bicarbonate, cholic acid and hippuric acid were decisively or considerably prophylactic, vitamin B_{12} and nicotinamide moderately effective, while vitamin B_1, anthranilic acid and cysteine were nearly and benzimidazole, sodium thiosulphate, cystine and norleucine utterly ineffective. Recently, Baumgarten[69] have reported that cysteine is effective in dithizone diabetes of rabbits at the dosis of 1,000 mg./kg., but we have not studied at the more dosis than 500 mg./kg..

(c) Against a diabetes induced by 8-hydroxyquinaldine, cystine, cysteine,

TABLE X

Results of Prevention against Alloxan Diabetes in Male Rabbits

Name of reagents	Dose	Body weight (g.) Before	Body weight (g.) Last	Experimental period (days)	Urine sugar	Blood sugar at the end of experiment (mg./dl.)	No. of cells in islet of Langerhans α-cells	No. of cells in islet of Langerhans β-cells
Normal (6 heads)		2000～2800			−	95±12	15.4	37.3
Control		2860	2620	4	±±±	338	16.9	5.4
		2360	2220	7	−～±±±	283	14.2	6.6
		2570	2300	7	±～±±±	280#	15.4	5.0
Benzimidazole	50 mg./kg.	2040	1290	6	±±±～−	160#§	15.6	7.2
		2000	1613	4	±±±	345	11.9	2.2
Sodium thiosulphate	1000 mg./kg.	2560	2260	7	−～±±±	299	23.4	16.1
		2230	1910	7	±～±±±	375	18.2	9.3
Sodium bicarbonate	1500 mg./kg.	2320	2240	7	−～±±	112	14.1	16.8
		2180	2230	7	−～±	120	14.4	22.5
		1800	1910	7	−～±	138	9.9	23.2
Vitamin B_1	400* mg./kg.	2060	2140	7	−～±	129	12.7	27.1
		2140	2290	7	+～−	140	16.0	23.4
Vitamin B_{12}	500 γ/kg.	1980	2020	7	±±～±±±	223	17.3	18.3
Nicotinamide	250 mg./kg.	2040		22hrs.	±±±	23†	15.1	11.5
		2380	2400	7	+～±±±	277	13.1	10.2
Anthranilic acid	400 mg./kg.	2260	2350	7	±±～±±±	238	14.3	16.8
		2150	2220	7	±±～−	215	12.9	14.3
Cystine	200 mg./kg.	2500	2510	7	±±～−	157	14.6	31.9
		2160	2180	7	−～+	89	13.9	29.6
Norleucine	500 mg./kg.	2150	2180	2	−～±	136†	15.1	9.9
Cholic acid	150 mg./kg.	2410	2400	7	−～±±±	161	14.4	14.0
		2230	2250	7	−～±±	189	16.3	19.9
Hippuric acid	300 mg./kg.	2140	2230	7	±±±～−	147	13.6	18.6
		2540	2550	7	±±±～−	101	12.0	10.9

* Injected subcutaneously 30 minutes before administration of alloxan.

\# Loss of appetite. † Weakness. § Diarrhea.

TABLE XI

Results of Prevention against Dithizone Diabetes in Male Rabbits

Name of reagents	Dose	Body weight (g.) Before	Body weight (g.) Last	Experimental period (days)	Urine sugar	Blood sugar at the end of experiment (mg./dl.)	No. of cells in islet of Langerhans α-cells	No. of cells in islet of Langerhans β-cells
Normal (6 heads)		2000～2800			−	95±12	15.4	37.3
Control		2200	2210	7	−～卌	422	14.0	4.8
		2180	2010	7	−～卌	411	15.5	4.1
Benzimidazole	50 mg./kg.	2220	1780	5	−～卌	410	20.9	5.6
		2440	2100	4	−～卌	395	11.9	2.2
Sodium thiosulphate	1000 mg./kg.	2080	1790	7	±～卌	305	17.5	6.5
		1990	1710	7	−～卌	345	19.2	4.5
Sodium bicarbonate	1500 mg./kg.	2210	1910	7	卌～−	95	15.9	17.3
		2320	2300	7	++～−	96	12.9	20.5
Vitamin B_1	400* mg./kg.	2150	2090	7	+～卌	303	16.0	7.9
		2330	2120	4	卌～+	123†	14.4	7.3
Vitamin B_{12}	750 γ/kg.	1900	1920	7	+～卌	321	12.2	12.7
Nicotinamide	250 mg./kg.	2370	2410	7	−～卌	95	11.6	13.8
		2440	2390	7	+～卌	296	13.8	11.2
Anthranilic acid	300 mg./kg.	2230	2280	7	−～卌	273	13.9	9.5
		2430	2420	7	−～卌	346	17.0	7.5
Cystine	300 mg./kg.	2240	2280	7	−～卌	305	15.1	5.5
		2160	2170	7	−～卌	396	13.7	6.3
Cysteine	500 mg./kg.	2380	2370	7	−～卌	270	16.2	8.9
Norleucine	500 mg./kg.	2100	2090	7	++～卌	296	15.8	5.6
Cholic acid	150 mg./kg.	2330	2390	7	卌～−	101	15.3	21.7
		2240	2290	7	++～−	111	14.2	19.2
Hippuric acid	300 mg./kg.	2180	2240	7	卌～++	142	15.6	10.2
		2450	2450	7	−～卌	289	13.4	9.9

* Injected subcutaneously 30 minutes before administration of dithizone.

† Weakness.

TABLE XII

Results of Prevention against 8-Hydroxyquinaldine Diabetes in Male Rabbits

Name of reagents	Dose	Body weight (g.) Before	Body weight (g.) Last	Experimental period (days)	Urine sugar	Blood sugar at the end of experiment (mg./dl.)	No. of cells in islet of Langerhans α-cells	No. of cells in islet of Langerhans β-cells
Normal (6 heads)		2000～2800			−	95±12	15.4	37.3
Control		2600	2510	7	±±±	419	12.0	7.1
		1990	1960	7	±±±	395	15.5	6.7
		2620	2560	7	±±±	342	13.4	5.2
Benzimidazole	50 mg./kg.	2340	2200	7	±±±～−	167	16.7	13.2
		2020	2000	7	±±±～−	143	20.0	15.3
Sodium thiosulphate	1000 mg./kg.	2120	2010	7	±±±～+	219‡	15.4	6.0
		2020	1800	7	+～±±±	346	18.2	5.6
Sodium bicarbonate	1500 mg./kg.	2200	2100	7	±±±～+	122	12.5	9.1
		2140	1980	7	±±±	215	14.2	12.6
Vitamin B_1	400* mg./kg.	2200	2210	7	±～±±±	251	13.4	11.0
		2400	2370	7	−～±±±	277	17.3	8.0
Vitamin B_{12}	500 γ/kg.	2100	2060	7	−～±±±	330	14.5	11.0
Nicotinamide	250 mg./kg.	2100	1970	7	−～±±±	288†	12.4	14.6
		2180	2210	7	+～±±±	275	14.3	13.7
Anthranilic acid	300 mg./kg.	2100	2060	7	+～±±±	166	13.0	9.7
		2250	2250	7	++～±±±	236	11.5	8.0
Cystine	200 mg./kg.	2540	2550	7	±±～−	120	12.7	13.2
		2210	2230	7	±±～−	110	14.3	24.6
Cysteine	400 mg./kg.	2200	2190	7	++～−	113	15.0	32.4
		2440	2480	7	±±～−	120	13.6	29.8
Cholic acid	150 mg./kg.	2460	2520	7	±±±～±	135	13.8	13.2
		2380	2350	4	±±±～±	227	14.5	10.1
Hippuric acid	300 mg./kg.	2550	2650	7	±±±～−	130	13.7	16.4
		2240	2290	7	±±±～−	128	13.9	14.8

* Injected subcutaneously 30 minutes before administration of 8-Hydroxyquinaldine.
‡ Loss of appetite. † Weakness.

TABLE XIII

Results of Experiments on Prevention of Oxine Diabetes in Guinea Pigs

Name of reagents	Body weight (g.) Before	Body weight (g.) Last	Sex	Dose (mg./kg.)	Methods of administration	Urine sugar	Blood sugar at the end of experi. (mg./dl.)	No. of cells in islet of Langerhans α-cells	No. of cells in islet of Langerhans β-cells
Normal (5 heads)	390 (320～510)					−	96±7	29.2 (21.4～33.6)	94.5 (81.0～106.9)
Control (6 heads)	375 (305～480)	363 (295～475)				+～卅	186 (110～225)	22.9 (20.4～28.7)	60.1 (34.3～78.6)
Vitamin B_1	330	350	♂	90×4	Subcut. inj. before 1 day, 90, 60, 30 minutes	−～±	117	18.1	79.5
	320	325	♂	120×4		±	121	35.9	101.1
Vitamin B_2	480		♂	15×4		*		29.3	36.2
	420	405	♂	〃		卄～卅	212	17.2	82.1
Vitamin B_6	475		♂	300×4		*		15.9	40.7
	430	440	♀	〃		−～+	180	35.4	96.1
Anthranilic acid	300	295	♂	200	Venous inj. before 30 minutes	±	120	18.1	76.1
	345	340	♂	300		−	143	20.4	65.8
Nicotinamide	405	410	♂	50		−～±	103	14.3	88.1
	495	480	♂	〃		−～±	115	16.5	86.9
Sodium thiosulphate	400	400	♂	200×4	Subcut. inj. before 1 day, 90, 60, 30 minutes	±～+	140	9.3	51.6
	450	465	♂	300×4		−	150	19.7	75.0
Atophane	330	340	♂	200	Venous inj. before 30 minutes	−	116	18.4	109.5
	380	385	♂	〃		～～±	128	26.4	112.7
Quinaldinic acid	375	400	♂	40	Subcut. inj. before 30 minutes	−～±	114	38.6	110.3
	470	475	♂	〃		−～±	122	39.4	126.9

* Urine sugar and blood sugar were not determined because animals were dead within 24 hours.

and benzimidazole proved decisively or considerably efficacious, cholic acid, hippuric acid and sodium bicarbonate moderately so, while the efficacy was very slight in vitamin B_1 and B_{12}, nicotinamide and anthranilic acid, and none in sodium thiosulphate.

Accurate comparison between results obtained with rats and rabbits would

be impracticable, since the dosis of alloxan is not the same, but generally speaking, it may be said that it is more difficult to prevent diabetogenesis by alloxan in rabbits than in rats. Among the tests with rabbits, prevention was most difficult against dithizone diabetes, and of the two other methods of diabetogenesis, the 8-hydroxyquinaldine diabetes seemed slightly more impervious to preventive action than alloxan diabetes.

With guinea pigs: The results of the experiments are shown in Table XIII. Vitamin B_1, nicotinamide, atophane, and quinaldinic acid showed preventive effect against the diabetes induced by oxine, and vitamin B_6 and anthranilic acid a slight preventive action, while vitamin B_2 and sodium thiosulphate were ineffective.

Results of experiments to explain the mechanism of prevention: Experiments conducted with the method described above and using 29 varieties of chemicals selected from the 38 items that proved effective in preventing alloxan diabetes in white rats, for determining whether their preventive reaction was effected within the pancreas or without it. The results obtained were as follows. (To avoid complications, we omit to go into detailed description of the results.)

1) The reagents whose action was clearly intra-pancreatic were benzimidazole, benztriazole, 5-nitrobarbituric acid, quinaldinic acid, sodium bicarbonate, bismuthiol I, sodium rhodizonate, anthranilic acid, cholic acid, ursodesoxycholic acid, hippuric acid, theobromine, dopa, tyrosine, cystine and norleucine, 16 items in total. All these are zinc-affine, except dopa and tyrosine.

2) As extra-pancreatic reactors we may enumerate borax, sodium thiosulphate, vitamin B_1, vitamin B_6 (aderoxin), vitamin B_{12}, folic acid, *p*-aminobenzoic acid, hesperidine, glutathione and cysteine, 10 items in all. None of these is zinc-affine.

3) The following showed both apparent intra- and extra-pancreatic actions: BAL, thiochrome and nicotinamide, of which BAL alone is zinc-affine.

Discussion

There are many substances reported to date as effective in preventing experimental diabetes mellitus, such as cysteine,[34,52] glutathione,[34] BAL,[54] thioglycolic acid,[34] sodium bisulfite,[49] *o*-phenylenediamine,[49] 3, 4-diaminotoluene,[49] adrenalin,[50] nicotinic acid,[56,59] nicotinamide,[56] 1, 2-dimethyl-4--amino-5-(d-1′-ribitylamino)-benzene,[59] 2-phenylquinoline-4-carboxylic acid (atophane),[59] pyridine dicarboxylic acid,[59] thiouracil,[52] 6-cyclopropyl-2--thiouracil,[52] 5-methyl-2-thiouracil,[52] 6-methyl-2-thiouracil,[52] 5-ethyl-2-thiouracil,[52] 2-thiohydrouracil,[52] methylene blue[62] (which Lazarow[57] has declared ineffective upon follow-up experiments), pentnucleotide[58] (which Houssay[52] has similarly declared ineffective), the majority being substances containing SH radicals or deemed to produce it in animal organism. We succeeded in adding to the list many items of preventive reagents, utterly unrelated to the substances reported in the past. Whereas the past researchers sought such prophylactics among the substances (1) known to react with alloxan in vitro, (2) presumed to

detoxicate alloxan in vivo (for example the substances containing SH radicals), (3) resembling qualitatively or in chemical constitution to the two types of substances above, and others, we extended our search, as mentioned above, into the realm of zinc-affine chemicals in general, acting upon the zinc theory of diabetes, and also of vitamins, especially of B group. And we were not disappointed in our expectations, for we found 24 of the 33 tested zinc-affine substances and 9 of the 14 tested vitamins clearly effective in prevention.

In looking over the list of the anti-diabetic zinc-affine substances, we find them to belong to a variety of chemical families, seldom to contain SH radicals in their constitution, and to be not ready to react with alloxan in vitro. Their efficacy is apparently not attributable to their capacity to detoxicate alloxan in vivo. The results of our experiments also revealed that their action was completed inside the pancreas. This limitation of their action to the intra-pancreatic seems to indicate that the action is in the last analysis " isletic ", that is, pertaining to the Langerhans islets, judging from what we know about the structure and the functions of the pancreas. This fact, in combination with the fact that the β-cells in the Langerhans islets are normally more richly provided than the cells of any other organs with histochemically provable zinc or zinc very ready to react and combine with zinc-affine reagents,[1] and the fact that the diabetogenic substances are all zinc-affine,[6,11] leads us to believe that these diabeto-preventive zinc-affine substances first combine with the zinc in the islets of Langerhans to effect the so-called blockade of the islets, so that the diabetogenic substances subsequently arriving at the islets are prevented from combining with the zinc in the islets, and the diabetes is prevented. Of course, the affinity to zinc inherent in the tested substances is variable from substance to substance, so when the affinity of the preventive substance is stronger or approximately equal to that of the diabetogenic such as alloxan, the preventive effect is manifested, but if the affinity of the diabetogenic is much above that of the tested reagent, we cannot expect an anti-diabetic effect to develop. That nine items of the tested zinc-affine substances showed no preventive efficacy is mainly attributable to such a cause.

The greater difficulty encountered in preventing dithizone diabetes rather than in preventing 8-hydroxyquinaldine and alloxan diabetes in rabbits is also presumably caused by the greater affinity of dithizone to zinc, in comparison with the other two diabetogenics.

In some cases in the experiments with rabbits on the prevention of alloxan diabetes with zinc-affine reagents, it was found that the effect was not so remarkable as with rats, and in some cases the effect was decidedly poorer. Such phenomena, at least partially, can be explained by the difficulty to administer the preventive reagents in a quantity large enough to match the large dosis of alloxan given to the animals in ratio to their greater body weight. Benzimidazole and anthranilic acid may be cited to represent such cases.

Among the 53 non-zinc-affine chemicals, 14 were found effective in preventing experimental diabetes, mainly substances containing SH radicals and vitamins of B group.

For the elucidation of the mechanism of prevention of diabetes by the substances containing SH radicals, cysteine, glutathione, BAL and sodium thiosulphate were specially tested and it was clarified that the effect was extra-pancreatic. BAL showed both intra- and exptra-pancreatic effects, and this may be explained by the fact of its being both zinc-affine and SH radicaled. It has been believed that substances with SH radicals have detoxicative effect against toxic substances, and our observation may be deemed well in consonance with the theory. It is highly probable that when alloxan is introduced in vivo glutathione is used up for the detoxication, so that by a previous dosis of glutathione or some substance having SH radical which is the effective principle for the detoxication, the alloxan is disposed of in the greater part before reaching the Langerhans islets and the diabetogenesis is effectively suppressed.

It is, however, premature to deduce the mechanism of diabetogenesis by alloxan from the experimental results showing reduction of glutathione in vivo after administration of alloxan and the prevention of the disease by means of large doses of glutathione, as something as follows, as the glutathione theory of diabetes will have it, that is, the β-cells are poorer in glutathione content than any other body cells so that when alloxan is introduced, these cells are promptly depleted of their glutathione supply, thus becoming defenseless against the injury by alloxan.

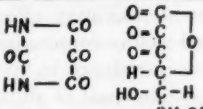
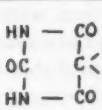
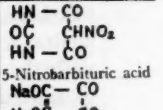
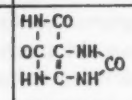
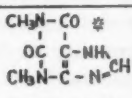
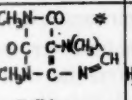
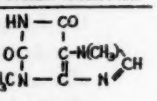
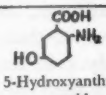
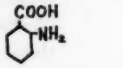
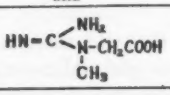
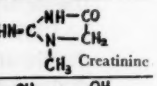
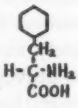
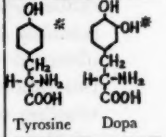
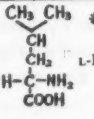
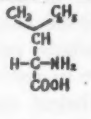
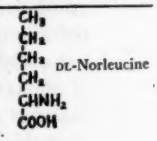
We must first point out that (1) there is no experimental proof that the β-cells are particularly poor in glutathione content, (2) that by experimental diabetogenesis by means of dithizone or some quinoline derivatives which presumably act in a similar diabetogenic process as alloxan, glutathione is not always observed to decrease immediately as shown in the experiments by Yui[70] of this laboratory, (3) that when preventive substances presumably incapable of reacting with alloxan in vivo (e.g., benzimidazole, cholic acid, vitamin B_{12}) are administered and the diabetes is effectively prevented, yet there is a marked reduction of glutathione content (Yui)[70], and (4) that glutathione content is naturally very low in baby rabbits, say, of 15 days after birth, and administration of alloxan is effective in reducing this low content to its lowest extreme, but no diabetes was nevertheless developed,[70] and we are led to conclude that the glutathione theory of diabetes is rather flimsily founded.

In the cases of vitamins and non-zinc-affine substances without SH radicals, the mechanism of diabeto-prevention is in all probability based either on a mediate or immediate reaction of the reagents with the diabetogenics in the blood or on a fortification of the action of detoxicative organs, such as the liver, but we are pursuing further verification on such points as would be deemed indispensable for judging the individual virtues of various substances.

Table XIV lists substances with resembling chemical formula but belonging to different categories of diabetogenic, preventive and neither diabetogenic nor preventive chemicals. The results of our experiments with 20 different chemicals and the results on diabetogenesis obtained by predecessors[3,4,36] and Okamoto and his collaborators[6,8,11] in their studies were duly consulted in compiling the list.

TABLE XIV

Chemical Formula of Diabetogenic,

		Diabetogenic	Non-diabetogenic, Non-preventive	Preventive
Alloxan and its allied formula substances and purine body	A	 Alloxan; Dehydroascorbic acid	 5,5-Dibromobarbituric acid	 5-Nitrobarbituric acid; Sodium rhodizonate
	B		 Uric acid;  Theophylline;  Caffeine	 Theobromine
Anthranilic acid etc.			5-Bromoanthranilic acid;  5-Hydroxyanthranilic acid	 Anthranilic acid
Amino acids	A		 Creatine	 Creatinine
	B		 DL-Phenylalanine	 Tyrosine; Dopa
	C		 L-Leucine;  Isoleucine	 DL-Norleucine

XIV

Preventive and their Allied Substances

Quinoline compounds

	Diabetogenic	Non-diabetogenic, Non-preventive	Preventive
A	Oxine; 8-Hydroxyquinaldine; 2,4-Dimethyl-X	8-Methoxyquinoline*; 8-Hydroxyquinaldinic acid; 4,8-Dihydroxyquinaldine*; 4-Hydroxy-8-methoxyquinaldine*; 5-Methyl-X; Yatren†; 8-Hydroxyquinoline-2-aldehyde; 5-Nitroquinaldinic acid; 5-Nitroso-X; 8-Nitroquinaldinic acid; 8-Hydroxy-2-(α-methyl)-quinolinemethanol	Xanthurenic acid; Quinaldinic acid; Atophane
B	5-Phenylazo-X; 5-(p-(or o-)-Tolylazo)-X; 5-(p-(or m-)-Hydroxyphenylazo)-X; 5-(p-Aminophenylazo)-X; 5-(p-Acetaminophenylazo)-X; 5-(p-Dimethylaminophenylazo)-X	5-(p-Nitrophenylazo)-X*; 5-(m,p-Diaminophenylazo)-X; 5-(p-Sulfophenylazo)-X; 5-(o-Carboxyphenylazo)-X; 5-(p-Arsonophenylazo)-X; 5-(p-Bromophenylazo)-X; Indoxine	
C	5-Phenylazo-Y; 5-(p-Hydroxyphenylazo)-Y; 5-(p-Dimethylaminophenylazo)-Y; 5-(p-Aminophenylazo)-Y; 5-(p-Acetaminophenylazo)-Y	5-(p-Tolylazo)-Y; 5-(p-Bromophenylazo)-Y; 5-(p-Nitrophenylazo)-Y*; 5-(p-Sulfophenylazo)-Y; 5-(p-Arsonophenylazo)-Y	

* non-zinc affine substances. † No experiment on prevention of diabetes, because it was dissolved in sodium bicarbonate which happens to show the preventive effect. X=8-hydroxyquinoline, Y=8-hydroxyquinaldine.

Patterson[36] opined that the -C-C-C- chain in the formula of alloxan has a meaning in its diabetogenic action and ascertained the diabetogenic action of dehydroascorbic acid which also contains such a chain, but we found that sodium rhodizonate, equally provided with a chain -C-C-C-C-, is not diabetogenic but diabeto-preventive.

Some author have commented that it is the part represented as -NH-CO--NH- in the chemical formula of alloxan that is important in the diabetogenesis (Griffiths)[71], but 5-nitrobarbituric acid is diabeto-preventive and 5, 5-dibromobarbituric acid is neither diabeto-genic nor -preventive, though both have the part.

Anthranilic acid is preventive, while 5-bromoanthranilic acid and 5-hydroxyanthranilic acid are effective neither in inducing nor preventing diabetes. The same may be said of the groups of creatinine—creatin, tyrosine—dopa—DL--phenylalanine and DL-norleucine—L-leucine—isoleucine.

The diabetogenic action of oxine, 8-hydroxyquinaldine, and 2, 4-dimethyl--8-hydroxyquinoline has been reported by Okamoto *et al.*,[6,11] but when the OH in the 8th position of their quinoline rings changes to OCH_3 or when as OH attaches to the 4th position, they lose their zinc-affinity and their diabetogenic and preventive effects. Substances with COOH group in their quinoline ring which do not lose their zinc-affinity but are nontoxic or only weakly toxic show preventive effect, as exemplified by xanthurenic acid, quinaldinic acid and atophane.

In short, in many cases, a small change in the chemical formula causes the substance to act diabetogenically or preventively, or altogether to lose the power to influence diabetogenesis, either positively or negatively.

Summary

1. Eighty-six varieties of chemical substances were tested for their efficacy in preventing alloxan diabetes in white rats and 37 items among them were found effective. 33 of the tested materials were zinc-affine substances, of which 24 were found diabeto-preventive. Of the remaining 53, which were non-zinc--affine, 14 proved to be effective in prevention.

2. Among the substances proved diabeto-preventive, we found some familiar to us in daily life and physiologically important, including 8 vitamins, mainly in B group, 6 varieties of cholic acids, theobromine, sodium bicarbonate and 7 kinds of amino acids.

3. The minimum effective doses and the duration of the effect were determined for all the preventive substances. The results showed that vitamin B_{12} had the strongest preventive effect, followed by benzimidazole, salicylaldoxime, sodium thiosulphate, BAL, nicotinamide, cholic acids and dopa.

4. The mechanism of diabeto-prevention was studied using 29 representative varieties of the substances found preventive, and the preventive action was found to be accomplished either intra-pancreatically, extra-pancreatically or

both intra- and extra-pancreatically.

5. Of the preventive substances, the zinc-affine varieties were found to act intra-pancreatically, probably intra-insularly, without exception—a finding apparently in support of Okamoto's zinc theory of diabetes mellitus.

6. The non-zinc-affine preventive substances (substances containing SH radicals and the majority of the effective vitamins) showed mechanism of extra--pancreatic prevention in most cases. The SH radicaled substances seemingly prevent diabetes by disposing of and detoxicating alloxan before it reached the pancreas. Such considerations also led us to doubt the tenability of Lazarow's glutathione theory of diabetogenesis.

7. With 12 of the preventive reagents as material, we conducted experiments for prevention of alloxan, dithizone and 8-hydroxyquinaldine diabetes in rabbits and found that the preventive effect was considerably impaired in some of the reagents. In general, the preventive action against alloxan diabetes is weaker in rabbits than in white rats. In some cases, the result may be explained by the lower doses of preventive reagents in ratio of the augmented doses of alloxan. Dithizone diabetes was found more difficult to prevent than the diabetes induced by the two other chemicals, which fact may be interpreted as due to the stronger affinity of dithizone with zinc.

8. Eight varieties of the substances found effective in preventing diabetes in white rats were found also effective in preventing experimental diabetes induced by oxine in guinea pigs in most cases.

9. Many substances with similar chemical formula and belonging to the same family of compounds were found to differ in their action—some being diabetogenic, some diabeto-preventive and the others neither diabetogenic nor preventive. In such cases, their manner of combination with zinc, that is, the fact that they are zinc-affine or not, was found to determine their reaction in regard to diabetes.

III. Studies on Rabbits from Ancestors Diabetic for Several Successive Generations, Especially on Spontaneous Occurrence of Diabetes in F_4 and F_5 Rabbits*

When alloxan,[3,4] dithizone,[6] oxine[6] and certain quinoline compounds[11,12,13,14] are administered, they will injure the β-cells in the Langerhans islets selectively, causing disturbance in the metabolism of carbohydrates in the body, leading to diabetes. It is, however, well known that in such a diabetic state, the metabolisms, not only of carbohydrates, but also of other substances, including fat and protein, are subject to certain changes. It may be conceived that when such a far-reaching disturbance in the metabolism of various substances happens the body, the cells in that organism, i.e., the somatic cells and the sexual cells, or maybe, the fertilized cells or the embryonic cells in the maternal body, are liable to some influence of the disturbance. In particular, we were interested in the question whether the sexual cells, the fertilized cells or the embryonic cells are liable to such influences, and if they are liable, what would be the nature of the influences, and were led to go into a detailed study on these points. To begin with, we made pairs of rabbits (P) diabetic with alloxan, and allowed them to breed after a fixed period. The youngs (F_1) were subjected to detailed histological examination of their bodily organs, especially the Langerhans islets in the pancreas, at various ages. We found that the α-cells in the islets showed little perceptible anomaly, but the β-cells were diminished in number and hypoplasied. Then, when the F_1 rabbits were again made alloxan-diabetic and their offsprings (F_2) were examined similarly, we came upon the fact that the β-cells in them were also decreased and hypoplasied, and indeed in a larger extent than in the F_1 rabbits.[12]

We thought it remarkable that, though there was no great difference in the severity of diabetes in P and F_1 rabbits, the Langerhans islets in the F_2 rabbits were more severely hypoplasied than those in F_1 rabbits. Such a phenomenon, however, seems to be hard understand through the knowledge of biology of present day, and may be in all probability deemed to belong to the category of permanent modification. At any rate, from the above findings, it is possible to make the following deduction. If we make diabetic generations after generations of some animals, the β-cells in the descendants will be more severely hypoplasied, so that a condition will be finally brought about, under which the insulin produced by such hypoplasied β-cells would not suffice to effect adequately the carbohydrate metabolism, and diabetes would be spontaneously (without taking such measures as administration of diabetogenic reagents) induced.

Acting upon such a presumption we proceeded to continue the experimental

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* By Kōzō OKAMOTO and Kin-ichirō FUKUTOME

diabetization for five successive generations and to examine the youngs ($F_1 \sim F_5$) histologically and biologically. At length, we have succeeded in finding spontaneous occurrence of diabetes in untreated young rabbits. A part of this study has been reported previously,[72,73] and we will here under give the outline of the study in whole.

Experimental

Materials and Method

Materials: (a) Animals. Adult and young rabbits procured from dealers (thus with no assurance if pure breed) were fed at this laboratory for more than 15 days, and 36 of these, which showed no anomaly in urine or blood sugar, were used in the experiments. 6 (♂ 3, ♀ 3) of them were used as control animals and the other 30 (♂ 12 and ♀ 18) in the experiments proper.

(b) Feeding. The rabbits were fed bean-cake refuse, unhulled oat or barley, carrots, sweet-potatoes, green vegetables and grass in sufficient quantity and freely, sometimes mixed with a little fish-meal. Enough water was supplied to pregnant, puerperal and diabetic rabbits. When diabetogenic reagents were used, oat and barley were excluded from the above diet to minimize the vitamin B content, which acted prophylactically against diabetes,[6,11,12] for the period of 1 to 2 weeks before the administration. After diabetes was successfully induced, the original diet was restored.

The young were kept for 35～50 days after birth with their mothers and kept apart thereafter. Often diabetic mother rabbits lacked the ability to milk the youngs, and in such cases, the youngs were given to other mothers (mostly mother rabbits in normal health) for lactation.

Method: In our study, rabbits were made diabetic, and after the lapse of a fixed period, paired and the begotten youngs normally bred, making the following tests in the meantime: (a) Measurement of bodyweight, test of sugar in urine and blood. (b) Thorough histological examination of entrails, especially Langerhans islets, of rabbits that died during the experiment or were sacrificed upon debilitation or of some of them upon attaining 120 days and one year after birth. (c) Some of young rabbits were made diabetic upon attaining maturity (generally after 150 days after birth) and then paired and made to breed as above.

By the repetition of such a process, we have arrived at the fifth generation (F_5) of diabetic rabbits. The outstanding test and research methods used in the study are as follows:

(1) Method of inducing diabetes: Alloxan (Eastman Kodak's alloxan monohydrate) was used, in 1～5% solution injected into the auricular veins of the rabbits. Our experience in the use revealed that even manufactures of the same works showed varied diabetogenic efficacy according to the dates of purchase and other circumstances, showing such a wide difference as 100～200 mg./kg. in the quantity required. In the course of our experiments, we used three brands of alloxan. In the first term, nearly covering the period of experimenting with

the P rabbits, the brand requiring 200 mg./kg. for inducing diabetes was used, and in the middle and later terms, the brands requiring 100 mg. or 120 mg./kg. were used.

As is well known, fluctuation in blood sugar is observed at the earlier stage following administration of such an agent. In P rabbits, which had to be given large quantities of alloxan, the drop of blood sugar content often became so vehement, that glucose had to be supplied. When the diabetes became too severe in its course, so that the rabbit lost appetite and began to excrete aceton urine, insulin was administered and care was taken to given an adequate supply of saline. As the rabbits too gravely diabetic frequently lacked ability of pairing and pregnancy, and also were apt to premature and still births or to produce hypoplasied youngs, in such cases, we took care to prevent aggravation of diabetes by insulin administration, so that the blood sugar was always kept below 350 mg./dl..

(2) Measurement of blood and urine sugar: Sugar in blood was measured by Hagedorn-Jensen's method and Benedict's reagent was used for measuring the sugar in urine (please refer to Chapter I).

When urine from F_4 and F_5 rabbits showed positive reaction to Benedict's reagent, without the rabbit being given alloxan, we frequently took care to ascertain the glucose content in urine by fermentation test and osazone generation reaction. The aceton compounds in urine were tested by Legal's method.

(3) Double charge test of sugar: Sakai *et al.*[74] of our laboratory have found that a sugar charge test as used with human subjects, when applied to rabbits, will give indeterminate results, and recommended the method given below for testing rabbits. We followed this method in our experiments.

The method: The rabbits tested are made to fast for 24 hours, then 0.5 g./kg. each of glucose is subcutaneously injected as 10% aqueous solution twice at an interval of 90 minutes. Samples of blood are taken 7 times, just before and at every 30 minutes after the injection and tested for blood sugar determination by Hagedorn-Jensen's method and for determination of Staub's effect.

(4) Histological examination: Formalin-fixed, paraffin-imbedded sections were stained with hematoxylin-eosin or by van Gieson's method, and the α-cells and β-cells of Langerhans islets were made discernible by Gomori's[19] staining. Thus, of the Langerhans islets, we not only have studied the histology of their constituent cells, but also the number of the cells per islet visible in a section, the size of the cells and islets and the number of islets.

(a) Number and size of cells in Langerhans islets. Sections were taken from the three parts of the pancreas near the spleen, the major curvature of the stomach and the duodenum and prepared into 4μ paraffin-imbedded sections stained by Gomori's method. 20～50 islets each were taken from the three parts at random, or 60～150 islets in total, and the number of α-cells and β-cells therein counted, to compute the average number per islet. Then, 30～100 α- and β-cells were taken from the islet in each part, totaling 90～300 cells, and the mean size of the cells determined with the help of an Abbe's drawing instrument and a planimeter.

(b) Number and size of Langerhans islets. 10~20 each of islets on 4μ paraffin-imbedded sections taken from the three parts of the pancreas and stained by Gomori's method, 30~60 islets in all, were measured and their mean area determined with an Abbe's drawer and a planimeter. Next a series of 500~1,500 paraffin-imbedded sections of 5μ thickness were prepared per pancreatic part and similarly stained, the number of the Langerhans islets found in them counted, and the total count of islets in the pancreas computed by the following equation. The mean of the number in the three parts was taken as the number of Langerhans islets in this study.

$$\frac{Z}{\frac{S}{r^2} \times D},$$

in which Z stands for the actual count of Langerhans islets in the sections, S for the total area of the examined sections in μ^2, D for the thickness of the sections (5μ in this study) and r for the magnification rate of the microscope.

By this formula, we get the numbers of islets per μ^3 of pancreas. These numbers are given in their equivalents in 1 mm^3 of pancreas in our tables.

(c) The volume of islets cells per 1 mm^3 of pancreas

It is difficult to ascertain the function of Langerhans islets with precision, but the approximate activity of them may be deduced from their morphological findings, namely, the histological figures, the number and the size of their cells. For this purpose, we have studied the number and the size of the cells and the islets, but it is difficult to arrive at a conclusion on the activity of the function by separate comparision of these factors. Thus, it is desirable to have one series of values for comparative estimation based on these several factors. For this object, we computed the volume occupied by the islet cells in 1 mm^3 of pancreas for comparison. This is very difficult to compute precisely, since the islets are variegated in shape, but we proceeded by assuming that the islets are spherical and derived the formula given below. Thus, we computed the tentative values of the volume to obtain data in estimating the activity of the islet cells in the rabbits.

$$V = \frac{4}{3\sqrt{\pi}} \times SN \times \sqrt{SN} \times I$$

where V represents the volume in μ^3 of the cells per mm^3 of pancreas
S the average area of a cell in μ^2
N the average number of cells per islet
I the number of islets per mm^3 of pancreas

Results

How the descendants of diabetic rabbits and the control rabbits were grown

A. Firstly, normal rabbits (♂ 3 and ♀ 3) were paired and the youngs F_1 and F_2 were used as control animals, as shown in Table XV. 40 F_1 and 10 F_2 rabbits were born, of which 27 were used in histological studies and 7 in other routine observations of urinal and blood sugar and double sugar charging tests.

B. Breeding of diabetic rabbits for several generations

(1) Breeding of F_1 rabbits (Table XVI): 12 male and 18 female rabbits were made diabetic with 200 mg./kg. of alloxan, but before pairing, 3 of the male and 7 of the female rabbits died, 3 males were incapable of copulation and 5 females were incapable of pregnancy of normal delivery, so that 6 each of male and female diabetic rabbits were successfully paired and 47 F_1 youngs were born. 17 of these died immediately or soon after birth, were killed by injury or sacrificed before being used as experimental materials (when more than 6 youngs were born from one mother and no nursing rabbits were found, the youngs per mother were limited to 5~6 each). Of the remaining 30, 10 were autopsied for histological examination after death or secrificing, 4 were used for routine observations and 15 were destined for alloxan diabetization to get F_2 rabbits after attaining maturity. Of these 15, 10 either died prematurely, or lost pairing, conceiving or normally parturiating capacity, only 5 becoming able to beget F_2 youngs.

(2) Breeding of F_2 rabbits (Table XVI): 2 males and 3 females of alloxan diabetic F_1 rabbits became the parents of 32 F_2 youngs. 4 of these were excluded from the use due to premature death and sacrificing and postmortem changes. 11 were used in histological examination, 5 for routine observations and 10 for alloxan diabetization. 5 only of the last 10 became the parents of F_3 rabbits.

(3) Breeding of F_3 rabbits (Table XVI): From 2 male and 3 female alloxan diabetic F_2 rabbits, 23 F_3 youngs were born. Of these 4 died prematurely, 6 were used for histological examination, 5 for routine observations and 10 were reserved for diabetization and breeding, 7 of which begot F_4 youngs.

(4) Breeding of F_4 rabbits (Table XVI): 43 F_4 youngs were born from 2 males and 5 females of alloxan diabetic F_3 rabbits. 5 died prematurely or by external injury, 4 were sacrificed for histological examination, 18 were used for routine observations and 10 were made alloxan diabetic. 4 of these became parents of F_5 youngs.

(5) Breeding of F_5 rabbits (Table XVI): From 2 each of male and female alloxan diabetic F_4 rabbits 24 youngs were born. 6 of these died, were sacrificed or injured to death before being usable in the experiments. 10 are being used for routine observations.

Growth of the rabbits

The bodyweights of the normal control rabbits and some of the alloxan diabetic F rabbits (marked with in Tables XV and XVI) bred as given above were measured for comparing their growth. The rabbits were weighed on the 30th~45th day after birth for the first time. The increase in their weights was as outlined in Table XVII. In all the groups, the number of the weighed rabbits is seen to decrease gradually. This is due to deaths, natural, by casualty or by sacrificing and by discontinuance of weighing upon alloxan diabetization. When a weighed rabbit became pregnant, it was excluded from the weighing during pregnancy and lactation.

Rabbits are deemed to mature sufficiently in 150~180 days after birth.

TABLE XV

Data on Normal Control Rabbits and their Parents

$\begin{cases} F_1 : 40 \text{ heads} \\ F_2 : 10 \text{ heads} \end{cases}$; as of March 31, 1954

Parents ♂ Animal No.	♂ Color of fur	♂ Urine sugar	♂ Blood sugar (mg./dl.)	Parents ♀ Animal No.	♀ Color of fur	♀ Urine sugar	♀ Blood sugar (mg./dl.)	No. of offsprings	Animal Number	Generation	Sex	Color of fur	Contents of experiment
1001	White	–	85	1002	White	–	91	6	12001#	F_1	♂	White	Died 129 days after birth
									12002#	〃	♀	〃	Under observation of progress
									12003#	〃	♂	〃	Used in other experiment
									12004#	〃	♂	〃	Killed 210 days after birth,‡
									12005#	〃	♂	〃	Killed 150 days after birth,‡
									12006#	〃	♀	〃	Used in other experiment
1003	White	–	78	1002	White	–	101	4	12007#	〃	♀	〃	Killed 1 year after birth,‡
									12008#	〃	♂	〃	Used in other experiment
									12009#	〃	♂	〃	Under observation of progress
									12010#	〃	♂	〃	〃
1004	White	–	82	1005	White	–	83	7	12011#	〃	♀	〃	〃
									12012#	〃	♂	White & brown	〃
									12013#	〃	♂	White	Died 111 days after birth
									12014#	〃	♂	〃	Killed 270 days after birth,‡
									12015#	〃	♂	〃	Killed 240 days after birth,‡
									12016#	〃	♂	〃	Killed 100 days after birth,‡
									12017#	〃	♂	〃	Killed 90 days after birth,‡
1004	White	–	90	1006	White	–	76	8	12018#	〃	♂	〃	Killed 120 days after birth,‡
									12019#	〃	♂	〃	〃 ,‡
									12020#	〃	♂	〃	Killed 60 days after birth,‡
									12021#	〃	♂	〃	Killed 180 days after birth,‡
									12022#	〃	♀	〃	Killed 60 days after birth,‡
									A	〃	♂	〃	Killed 3 days after birth
									B	〃	♂	〃	
									C	〃	♀	〃	
1001	White	–	93	1002	White	–	82	9	12023	〃	♂	〃	Killed 5 days after birth,‡
									12024	〃	♂	〃	〃 ,‡
									12025	〃	♀	〃	Killed 10 days after birth,‡
									12026	〃	♂	〃	〃 ,‡
									12027	〃	♂	〃	Killed 20 days after birth,‡
									12028	〃	♀	〃	〃 ,‡
									12029	〃	♂	〃	Killed 30 days after birth,‡
									12030	〃	♂	〃	Killed 40 days after birth,‡
									12031	〃	♀	〃	Killed 50 days after birth,‡
1003	White	–	100	1006	White	–	82	6	12032	〃	♂	〃	Killed 70 days after birth,‡
									12033	〃	♂	〃	〃 ,‡
									12034	〃	♀	〃	Killed 80 days after birth,‡
									12035	〃	♂	〃	Killed 120 days after birth,‡
									12036	〃	♀	〃	Killed 150 days after birth,‡
									12037	〃	♂	〃	Killed 210 days after birth,‡
12003	White	–	80	12007	White	–	76	7	12123#	F_2	♀	〃	Died 181 days after birth
									12124#	〃	♂	〃	Died 213 days after birth
									12125#	〃	♂	〃	Under observation of progress
									12126#	〃	♀	〃	Used in other experiment
									A'	〃	♀	〃	Killed 2 days after birth
									B'	〃	♂	〃	
									C'	〃	♂	〃	
12012 White & brown	–	–	93	12002	White	–	79	3	12127	〃	♀	〃	Died 253 days after birth
									12128	〃	♀	〃	Under observation of progress
									D'	〃	♂	White & brown	Killed 3 days after birth

Rabbits measured for body weight more than once every week. The blood sugar values show before mating. ‡ Histological examination was performed.

TABLE XVI

Data on F Rabbits and their Parents (as of March 31, 1954)

Generation	Parents								Content of experiment					
	♂				♀				Animal					
	Animal No.	Color of fur	Urine sugar	Blood sugar (mg./dl.)	Days between onset of diabetes and pairing	Animal No.	Color of fur	Urine sugar	Blood sugar (mg./dl.)	Days between onset of diabetes and pairing	No. of offsprings	Number	Sex	Color of fur	Mode of experiment	Dose of administration of alloxan (mg./kg., intravenously)	Days after birth to production of diabetes
heads)	1020	W	###	240	15	1017	W	###	245	277	9	11115‡	♀	"	Alloxan diabetes produced	50, 70	215, 240
												11116‡	♂	"	Killed 71 days after birth,‡		
												11117‡	♂	"	Died 46 days after birth,‡		
												11118‡	♀	"	Died 60 days after birth,‡		
												11119‡	♂	"	Died 78 days after birth,‡		
												11120‡	♂	"	Killed 120 days after birth,‡		
	W	W	###	252	20	1017	W	###	238	31	2	11113	♀	W	Died 70 days after birth,‡		
												11114	♂	"	Died 50 days after birth,‡		
	1011	W	###	248	18	1014	B-G	###	230	180	5	11101	♂	W	Alloxan diabetes produced	150	136
												11102	♂	B-G	"	150	167
												11103	♀	"	Died within 1 month after birth		
												11104	♀	W			
												11105	♀	"			
	1012	B-G	###	260	38	1016	W	###	200	10	7	11106	♀	"	Alloxan diabetes produced	150	184
												11107	♂	B-G	"	150	192
												11108	♀	W			
												11109	♀	"			
												11110	♀	"			
												11111	♀	"	Died within 1 month after birth		
												11112	♀	B-G			

heads)

F_1 (47

1013	W	###	280	95	1026	Dark-B	###	264	71	13	11132#	♂	B	Killed 120 days after birth,‡		
											11133#	♀	W	Alloxan diabetes produced	70	121
											11134#	♂	″	″	70	136
											11135#	♂	″	Died 91 days after birth,‡		
											11136#	♀	″	Alloxan diabetes produced	100	120
											11137#	♀	″	″	70	121
											11138#	♀	″	″	100	136
											11139	?	?	Killed immediately after birth		
											11140	?	″			
											11141	?	″			
											11142	?	″			
											11143	?	″			
											11144	?	″			
1021	W	###	232	917	1023	W	##	230	322	4	11121#	♀	W	Died 127 days after birth,*		
											11122#	♂	″	Alloxan diabetes produced	90	130
											11123#	♂	″	Under observation of progress		
											11124#	♀	″	″		
1022	W	###	308	664	1024	W	###	320	194	10	11125#	♂	″	Alloxan diabetes produced	90	160
											11126#	♂	″	Under odservation of grogress		
											11127#	♀	″	″		
											11128#	♀	″	Alloxan diabetes produced	90	160
											11129#	♂	″	Killed 120 days after birth,‡		
											11130	♂	″	Alloxan diabetes produced	80, 85	155, 163
											11131	♀	″	″	80	155
											A	?	?	Killed 1 day after birth		
											B	?	″			
											C	?	″			
11107	B-G	###	243	40	11106	W	###	260	46	7	11201	?	W	Killed 5 days after birth		
											11202#	♂	″	Alloxan diabetes produced	50, 60	113, 133
											11203#	♀	″	″	100	105
											11204#	♀	B-G	″	50, 70	113, 133
											11205#	♂	″	″	100	105
											11206#	♀	″	″	70	110
											11207#	♂	″	Killed 120 days after birth,‡		
11107	B-G	###	260	39	11102	B-G	###	270	39	6	11208	♂	″	Died 60 days after birth,‡		
											11209	♀	″	Died 48 days after birth,‡		
											11210#	♀	″	Killed 120 days after birth,‡		
											11211#	♂	″	Killed 68 days after birth,‡		

Parents ♂	Parents ♀	Generation	Animal No.	Animal Color of fur	Animal Sex	Animal Number	No. of offsprings	Content of experiment: Mode of experiment	Dose of administration of alloxan (mg./kg., intravenously)	Days after birth to production of diabetes
					♂ ♀	11212# 11213#		Alloxan diabetes produced	70, 60 70, 70	110, 275 112, 176
		F_2 (32 heads)	11107	B-G	♂	11214#	7	"	70	112
					♀	11215#		"	70	187
					♂	11216#		Killed 120 days after birth,‡		
					♂	11217#		Died 49 days after birth,‡		
					♂	11218		Died 37 days after birth,‡		
					♂	11219		Died 39 days after birth,‡		
					♀	11220#		Alloxan diabetes produced	50	191
					♀	11221#	7	Died 90 days after birth,*		
					?	11222		Died 50 days after birth,*		
					?	11223#		Died 58 days after birsh,*		
					♂	11224		Died 58 days after birth,*		
					♂	11225#		Killed 1 year after birth,‡		
					♂	11226		Died 28 days after birth,‡		
					?	11227		Died immediately after birth		
					♂ ♀	11228#	5	Under observation of progress		
					♂	11229#		"		
					♀	11230#		"		
					♂	11231#		"		
					♀	11232#		"		
					♂	11360#		Alloxan diabetes produced	70, 75	153, 195

Parents ♂					Parents ♀				
Animal No.	Color of fur	Urine sugar	Blood sugar (mg./dl.)	Days between onset of diabetes and pairing	Animal No.	Color of fur	Urine sugar	Blood sugar (mg./dl.)	Days between onset of diabetes and pairing
11130	W	###	252	101	11131	W	###	245	101
11107	B-G	###	240	196	11106	W	###	240	202
11107	B-G	###	275	112	11106	W	###	245	118

F_3 (28 heads)	11206	B-G	卌	309	95	11213	B-G	卌	245	70	5	11361‡	♀	B-G	〃	70, 50, 60, 70	120,405,446,469
												11362‡	♀	〃	〃	60, 60	125, 139
												11363‡	♂	〃	Under observation of progress		
												11364‡	♀	〃	〃		
	11202	W	卌	302	87	11213	B-G	卌	262	26	6	11365‡	♂	W	Killed 150 days after birth,‡		
												11366‡	♂	〃	Killed 240 days after birth,**		
												11367‡	♀	B-G	Died 150 days after birth,‡		
												11368‡	♂	〃	Under observation of progress		
												11369‡	♂	W & B-G	Alloxan diabetes produced	60, 50, 65, 70	169,572,613,638
												11370	♀	〃	Died 13 days after birth		
	11202	W	卌	280	107	11215	W	卌	357	49	4	11371	♂	W	Died 17 days after birth		
												11372‡	♀	〃	Alloxan diabetes produced	60, 50	237, 424
												11373‡	♂	〃	Killed 240 days after birth,‡		
												11374‡	♂	〃	Lost (300 days after birth)		
	11202	W	卌	280	107	11204	B-G	卌	265	102	7	11375‡	♂	W & B-G	Killed 120 days after birth,‡		
												11376‡	♀	〃	Alloxan diabetes produced	70, 75	133, 223
												11377‡	♀	B-G	〃	70,60,75,70,70	146, 158, 164, 182, 438
												11378‡	♂	W	Killed 1 year after birth,‡		
												11379‡	♀	〃	Alloxan diabetes produced	70	133
												11380‡	♂	〃	Died 210 days after birth,‡		
												11381‡	♂	〃	Alloxan diabetes produced	80	133
	11202	W	卌	295	121	11215	W	卌	380	209	6	11382‡	♂	〃	Died 131 days after birth,*		
												11383‡	♂	〃	Under observation of progress		
												11384‡	♀	〃	Alloxan diabetes produced	60	180
												11385‡	♂	〃	Under observation of progress		
												11386	♂	〃	Died 20 days after birth		
												11387	?	?	Died 18 days after birth		
	11369	W & B-G	++~卌	205	17	11361	B-C	++~卌	190	64	5	11401	♂	W	Died 27 days after birth		
												11402‡	♂	〃	Alloxan diabetes produced	60, 70	208, 282
												11403‡	♂	〃	Died 103 days after birth,*		
												11404‡	♂	W & B-G	Died 189 days after birth,*		
												11405‡	♂	B-C	Under observation of progress		
	11369	W & B-G	++~卌	180	18	11376	W & B-G	卌	275	19	4	11406‡	?	W & B-G	Died 36 days after birth,*		
												11407‡	♀	W	Alloxan diabetes produced	60, 70, 70	206, 239, 249
												11408‡	♂	W & B-G	Under observation of progress		

Generation	Parents								Content of experiment					
	♀					♂			Amimal					
	Animal No.	Color of fur	Urine sugar	Blood sugar (mg./dl.)	Days between onset of diabetes and pairing	Animal No.	Color of fur	Urine sugar	Blood sugar (mg./dl.)	Days between onset of diabetes and pairing	No. of offsprings	Number	Sex	Color of fur	Mode of experiment	Dose of administration of alloxan (mg./kg., intravenously)	Days after birth to production of diabetes
												11409#	♂	W & B-G	Died 120 days after birth,†		
F_1 (43 heads)	11381	W	###	245	275	11384	W	###	256	103	2	11427# 11428#	♀ ♂	〃 〃	Died 151 days after birth,§ Under observation of progress	〃	
	11381	W	###	260	233	11384	W	###	289	61	4	11423# 11424# 11425# 11426#	♂ ♀ ♀ ♀	W 〃 〃 〃	Under observation of progress Died 146 days after birth,§ Under observation of progress 〃		
	11381	W	###	245	194	11377	B-G	##～#	185	177	7	11418# 11419# 11420# 11421# 11422# A B	♀ ♂ ♀ ♀ ♂ ♀ ♂	〃 〃 〃 〃 W & B-G ? ?	Alloxan diabetes produced Killed 120 days after birth,† Alloxan diabetes produced 〃 〃 Killed immediately after birth 〃	65 65 65 65	241 266 266 241
	11381	W	###	250	54	11377	B-G	###	243	5	5	11410# 11411# 11412# 11413# 11414#	? ♂ ♂ ♀ ♀	W 〃 B-G W & B-G	Died 43 days after birth,* Alloxan diabetes produced Killed 240 days after birth,† Alloxan diabetes produced 〃	60, 65 60, 60 60	189, 197 164, 172 153
	11381	W	###	286	125	11372	W	###	235	43	3	11415# 11416# 11417#	♂ ♀ ♂	W 〃 〃	Under observation of progress Alloxan diabetes produced Under observation of progress	65, 70, 70	124, 143, 152

F_5 (24 heads)	11381	W	‡‡	253	282	11377	B-G	‡‡	240	9	7	11429#	♀	B-G	〃		
												11430#	♂	W & B-G	〃		
												11431#	♂	〃	〃		
												11432#	♂	W	〃		
												11433#	♂	〃	Killed 120 days after birth,‡		
												11434#	♂	〃	Under observation of progress		
												11435#	♂	〃	Died 85 days after birth,*		
	11381	W	‡‡	267	338	11384	W	‡‡	265	166	6	11436#	♂	〃	Under observation of progress		
												11437#	♂	〃	Died 50 days after birth,*		
												11438#	♂	〃	Under observation of progress		
												11439#	♂	〃	〃		
												C	?	?	Killed by rat or senior rabbit		
												D	?	?	〃		
	11411	W	‡‡	265	24	11407	W	‡~‡‡	190	5	7	11503#	♀	W	Under observation of progress		
												11504#	♂	〃	〃		
												11505#	♀	〃	Died 85 days after birth,*		
												11506#	♂	〃	Under observation of progress		
												11507#	♀	〃	〃		
												11508#	♀	〃	Died 119 days after birth,§,*		
												11509#	♀	〃	Died 134 days after birth,§,‡		
	11411	W	‡~‡‡	193	5	11413	B-G	‡‡	221	24	7	11510#	♂	〃	Under observation of progress		
												11511#	♀	B-G	〃		
												11413F	♀	W	Killed 10 days after birth		
												A	?	B-G	Fed upon 3 days after birth		
												B	?	〃			
												C	?	〃			
												D	?	W			
	11402	W	‡‡	293	107	11413	B-G	‡‡	214	132	6	11512#	♂	B-G	Under observation of progress, 73rd day		
												11513#	♂	〃	Died 57 days after birth,*		
												11514#	♀	W	Died 47 days after birth,*		
												11515#	♀	W & B-G	Under observation of progress, 73rd day		
												11516#	♂	W	Died 48 days after birth,*		
												11517	♂	〃	Died 15 days after birth		
	11402	W	‡‡	275	109	11407	W	‡‡	240	139	4	11518#	♀	〃	Under observation of progress, 71st day		
												11519#	♂	〃	〃 ,		
												11520#	♂	〃	Died 68 days after birth,*		
												11521#	♀	〃	Died 52 days after birth,*		

Rabbits measured for body weight more than once every week. The blood sugar values show before mating. ‡ Histological examination was performed. * Histological examination abandoned due to advanced post-mortem degeneration. ** Detailed examination of Langerhans islets abandoned due to pancreatic cirrhosis. B Brown, G Gray, W White. § Died during observation of progress.

TABLE XVII

Body Weight of Normal and F Rabbits Made Diabetic for Successive Generations (g.) (under 1 year after birth, as of March 31, 1954)

Generation / No. of days after birth / Total No.	Normal rabbits		Offsprings of rabbits diabetic for successive generations				
	F_1	F_2	F_1	F_2	F_3	F_4	F_5
	22	6	22	23	24	38	18
30	629 (400 ~1100) [21]	687 (400 ~860) [6]	458 (380 ~560) [13]	569 (430 ~650) [19]	986 (820 ~1100) [7]	753 (540 ~960) [26]	759 (530 ~1080) [12]
45	938 (600 ~1560) [22]	870 (470 ~1180) [6]	812 (450 ~1010) [22]	906 (600 ~1050) [16]	1094 (800 ~1340) [10]	988 (630 ~1400) [38]	1080 (820 ~1520) [16]
60	1190 (690 ~1640) [22]	1057 (530 ~1390) [6]	1015 (800 ~1250) [21]	1138 (730 ~1400) [23]	1137 (700 ~1540) [20]	1231 (710 ~1920 [37]	1263 (1020 ~1500) [16]
90	1686 (1100 ~2140) [20]	1491 (1025 ~1950) [6]	1564 (1000 ~1920) [17]	1665 (1160 ~2120) [20]	1451 (960 ~2000) [24]	1731 (1160 ~2430) [36]	1806 (1390 ~2000) [14]
120	2069 (1660 ~2400) [16]	2222 (1900 ~2450) [6]	2157 (1840 ~2450) [17]	2090 (1750 ~2630) [18]	1870 (1360 ~2450) [24]	2318 (1820 ~2450) [33]	2240 (1780 ~2750) [10]
150	2397 (2100 ~2630) [12]	2593 (2080 ~2900) [6]	2413 (2180 ~2700) [7]	2456 (2000 ~2800) [5]	2011 (1760 ~2460) [16]	2720 (2230 ~3500) [27]	2590 (2150 ~3080) [8]
180	2633 (2200 ~3070) [11]	2730 (2250 ~3050) [6]	2768 (2390 ~3220) [4]	2690 (2420 ~2930) [5]	2211 (1800 ~2780) [12]	3034 (2340 ~3920) [21]	2913 (2600 ~3260) [8]
210	2805 (2520 ~3300) [11]	2796 (2200 ~3250) [5]	3007 (2730 ~3390) [3]	2875 (2720, 3030) [2]	2252 (1700 ~3040) [11]	3240 (2450 ~3970) [18]	2968 (2740 ~3500) [5]
240	2994 (2400 ~3470) [10]	3032 (2430 ~3450) [4]	3485 (3270 ~3670) [4]	2980 (2560 ~3090) [3]	2371 (1900 ~3340) [9]	3344 (2300 ~4000) [16]	3180 (3100, 3260) [2]

No. of days after birth \ Generation / Total No.	Normal rabbits		Offsprings of rabbits diabetic for successive generations				
	F_1	F_2	F_1	F_2	F_3	F_4	F_5
	22	6	22	23	24	38	18
270	3241 (2390 ~3580) [8]	3260 (3120, 3400) [2]	3445 (3030, 3860) [2]	2935 (2670, 3200) [2]	2493 (2000 ~2960) [7]	3378 (2400 ~4000) [11]	
300	3356 (3210 ~3700) [5]	3570 (3320, 3820) [2]	3505 (3200, 3810) [2]	2820 [1]	2591 (2190 ~3120) [6]	3524 (2500 ~4000) [7]	
330	3474 (3170 ~3920) [5]	3310 (2620, 4000) [2]	3310 [1]	2920 [1]	2618 (2180 ~3130) [6]	3566 (2530 ~4000) [7]	
365	3502 (3200 ~4000) [5]	3950 (3950, 4000) [2]		2640 [1]	2707 (2350 ~3030) [6]	3395 (2500 ~4000) [4]	

The figures in brackets show the number of rabbits.

The increase of bodyweight up to the maturity shows no significant difference between the normal and alloxan diabetic F rabbits in all generations, except that the F_3 rabbits were somewhat slow in growth but the F_4 and F_5 rabbits were seemingly slightly in better growth. After 210 days, the decrease of cases becomes so high in some groups that comparative study grows more and more difficult but the above tendency is found to prevail. We cannot think of any clear reason why the F_3 rabbits are generally lighter. Among the F_4 and F_5 rabbits, there were many that became spontaneously diabetic, as will be detailed hereunder. These showed an abrupt increase in bodyweight between around 60 and 120 days after birth and this seems to be the factor that resulted in the larger bodyweight of F_4 and F_5 rabbits as a group than in other groups and the control rabbits.

Results of histological examination and measurement of Langerhans islets

The pancreas of the rabbits that were sacrificed or otherwise were extracted immediately after their death (the pancreas of rabbit taken out after some time from the moment of death were not fit for examination) were prepared for microscopic examination by Gomori's method. Rabbits of 120 days after birth, when, we take, they are in the first stage of maturation, were subjected to most thoroughgoing examination. In such studies, rabbits of less than 2 kg. in bodyweight on account of diarrhea etc. were excluded as below normal in growth.

TABLE

Findings on Langerhans Islets

		No. of days after birth	5	10	20	30	40	50	60
Materials	Animal No.	Normal	12023, 12024	12025, 12026	12027, 12028	12029	12030	12031	12020, 12022
		F_1						11117, 11114	11118
		F_2				11226	11218, 11219	11217, 11209	11208
		F_3							
		F_4				11401	11406		
	Body weight (g.)	Normal	103 (115, 90)	131 (108,153)	260 (280,240)	506	720	635	915 (1050, 780)
		F_1						675 (450, 900)	1100
		F_2				500	705 (700, 710)	870 (830, 910)	1300
		F_3							
		F_4				710	830		
α-cells	No. of cells	Normal	4.7 (4.8, 4.5)	5.3 (5.0, 5.5)	5.9 (5.9, 5.8)	8.2	8.5	8.8	6.3 (6.3, 6.2)
		F_1						6.6 (5.6, 7.5)	7.0
		F_2				6.0	5.7 (5.3, 6.1)	7.1 (8.6, 5.6)	6.8
		F_3							
		F_4				4.3	5.5		
	Size of cells (μ^2)	Normal	133.1 (133.0, 133.2)	132.9 (133.1, 132.7)	141.3 (141.3, 141.3)	133.1	137.8	129.0	142.1 (142.7, 141.5)
		F_1						126.6 (119.4, 133.8)	129.8
		F_2				83.5	88.7 (87.6, 89.7)	102.8 (106.3, 99.3)	112.6
		F_3							
		F_4				77.1	78.1		

XVIII

of Normal and F Rabbits

70	80	90	100	120	150	180	210	240	270	365
12032, 12033	12034	12017	12016	12035,12018,12019	12005, 12036	12021	12004, 12037	12015	12014	12007
11116, 11113	11119	11135		11120,11132,11129						
11210				11207,11210,11216						11225
				11375	11365, 11367		11380	11373		11378
				11409,11419,11433				11412		
1385 (1270, 1500)	1690	1640	1800	2137 (2100, 2000, 2310)	2200 (2300, 2100)	2100	2370 (2400, 2340)	2570	2320	3200
1330 (1120, 1540)	1200	1540		2167 (2050, 2350, 2100)						
1400				2127 (2230, 2150, 2000)						2640
				2200	2230 (2000, 2460)		2120	2220		3030
				2093 (2050, 2000, 2230)				2930		
8.3 (9.6, 7.0)	7.2	8.3	10.2	7.4 (7.3, 7.5, 7.4)	9.9 (9.9, 9.8)	11.1	12.2 (12.0, 12.4)	12.2	13.1	13.3
8.6 (8.2, 8.9)	7.2	9.7		9.3 (10.3, 8.2, 9.4)						
7.9				10.9 (10.8, 11.0, 11.0)						13.5
				9.1	9.3 (8.7, 9.9)		8.3	8.0		15.6
				7.5 (7.4, 7.4, 7.7)				9.0		
133.1 (141.5,129.3)	130.0	131.6	126.8	147.8 (135.0,149.8,158.5)	145.8 (148.1, 143.4)	148.4	143.1 (146.1, 140.1)	172.6	164.5	167.0
113.8 (108.6,119.0)	112.4	120.4		128.5 (126.9,130.2,128.3)						
98.1				126.4 (124.3,132.4,122.6)						122.2
				120.1	114.4 (119.8, 109.0)		116.2	108.5		121.1
				110.8 (121.4,107.1,104.1)				116.7		

		No. of days after birth	5	10	20	30	40	50	60
β-cells	No. of cells	Normal	18.6 (22.1, 15.1)	19.9 (20.0, 19.7)	24.3 (26.0, 22.6)	29.3	27.9	27.7	26.0 (26.2, 25.8)
		F_1						26.7 (27.2, 26.1)	28.3
		F_2				21.3	24.3 (23.8, 24.7)	25.4 (26.0, 24.8)	24.3
		F_3							
		F_4				23.3	19.3		
	Size of cells (μ^2)	Normal	134.7 (147.5, 121.9)	155.5 (155.4, 155.5)	141.9 (148.6, 135.2)	142.5	138.7	148.3	147.9 (148.0, 147.7)
		F_1						131.7 (127.5, 135.9)	132.5
		F_2				104.1	104.3 (104.9, 103.7)	109.2 (109.5, 108.9)	125.3
		F_3							
		F_4				90.7	102.5		
Size of islets (100 μ^2)		Normal	43.0 (44.2, 41.8)	53.0 (54.1, 51.9)	45.6 (48.3, 42.9)	47.8	52.0	55.3	63.9 (63.5, 64.3)
		F_1						48.0 (47.1, 48.9)	53.0
		F_2				34.7	38.0 (40.0, 36.0)	46.0 (46.0, 45.9)	60.2
		F_3							
		F_4				32.5	31.7		
No. of islets per 1 mm³ of pancreas		Normal	43.8	76.6	50.0	44.7		36.9	29.3
		F_1							
		F_2							
		F_3							
		F_4							

70	80	90	100	120	150	180	210	240	270	365
30.2 (27.2, 33.2)	30.0	28.4	28.2	31.4 (30.4, 32.6, 31.1)	35.0 (36.7, 33.3)	35.9	38.4 (38.8, 38.0)	38.7	38.1	39.0
31.0 (32.9, 29.0)	25.4	25.4		27.2 (29.1, 26.1, 26.5)						
27.6				22.5 (21.5, 22.6, 23.5)						24.3
				16.0	16.8 (16.4, 17.1)		15.9	22.7		22.1
				13.3 (12.9, 13.7, 13.3)				18.1		
144.6 (146.3,142.9)	152.4	146.1	160.1	163.2 (154.3,166.5,168.9)	154.8 (162.8, 146.7)	174.6	164.8 (168.7, 160.9)	183.9	196.9	184.3
125.5 (117.6,133.4)	132.2	132.2		142.3 (142.7,145.6,138.5)						
119.0				130.1 (138.4,124.9,126.9)						123.7
				129.2	131.2 (130.2, 132.2)		128.8	116.1		125.0
				103.5 (108.6,97.7, 104.1)				125.9		
61.8 (62.5, 61.0)	61.0	68.4	70.5	79.3 (76.6, 79.3, 82.0)	81.6 (87.5, 75.7)	94.5	97.1 (103.4, 90.8)	107.8	102.5	99.0
56.5 (55.9, 57.0)	49.0	54.3		74.2 (80.5, 68.1, 73.9)						
53.6				68.6 (68.3, 65.5, 72.1)						63.2
				62.8	61.2 (64.8, 57.5)		53.1	52.5		60.3
				47.1 (45.0, 49.3, 46.9)				50.8		
31.8	32.5	27.5	29.0	30.7 (25.9, 32.9, 33.3)	27.1	28.0	23.3	16.3	15.7	16.2
				25.9 (25.2, 26.7)						
				30.0 (30.0, 22.3, 37.7)						29.6
				17.9	24.7 (18.0, 31.3)		25.8	21.9		
				29.0*						

Volume of cells per mm³ of pancreas (10,000 μ^3)		No. of days after birth	5	10	20	30	40	50	60
	α-cells	Normal	53	107	90	117		106	59
		F_1							
		F_2							
		F_3							
		F_4							
	β-cells	Normal	613	988	903	963		729	533
		F_1							
		F_2							
		F_3							
		F_4							

*=Value of No. 11409. ‡=Value when islet number is taken at 29.

(1) Measurements on Langerhans islets in normal rabbits

The following values were obtained by Fukutome *et al.*[75] in the laboratory of Okamoto as the results of their experiments on the subject, and these values were used in reference in our study.

(a) Number of α-cells: The number of these cells is small in the earliest period after birth, standing at about 5 per islet, increasing gradually to 12 on around 180～210th days and remaining at 12～13.3 in average thereafter.

(b) Number of β-cells: These are also few shortly after birth, numbering around 20 per islet, but gradually increasing to 38 by the 210th day, remaining on the level of 38～39 thereafter.

(c) Size of the cells: Both the α- and β-cells are small in the early stage after birth, about 130 μ^2 in the area they occupy on the sections, but grow slowly to 145 and 165 μ^2 on the 210th day and to 170 and 185 μ^2 on the 240th day, keeping these sizes ever afterwards.

(d) Size of the islets: About $43 \times 100\,\mu^2$ at birth, growing up to around $100 \times 100\,\mu^2$ on the 210th day, which size is permanently kept thereafter.

(e) Number of islets: The number of islets per mm³ of pancreas is very high at birth, amounting to about 50, but diminishes gradually to 23 on the 210th and to 16 on the 240th day, remaining these thereafter.

70	80	90	100	120	150	180	210	240	270	365
65	71	72	94	87 (64, 94, 102)	115	141	132	110	97	128
				82 (95, 69)						
				112 (107, 93, 137)						149
				44	64 (46, 82)		59	42		
				59‡						
789	791	551	661	817 (539, 970, 942)	942	1047	917	793	780	743
				487 (505, 468)						
				358 (366, 252, 456)						368
				137	194 (134, 253)		284	223		
				114‡						

(f) The volume occupied by α- and β-cells in 1 mm^3 of pancreas: As shown in the Table, it ranges from 53 to $141 \times 10{,}000\,\mu^3$ by α-cells and from 613 to $1{,}047 \times 10{,}000\,\mu^3$ by β-cells, in most cases from 70 to $130 \times 10{,}000\,\mu^3$ in the former and from 650 to $900 \times 10{,}000\,\mu^3$ in the latter. Thus, as seen above, the number and the size of the cells and the islets are considerably varied according to the days after birth of the rabbit but the volume occupied by the cells in total computed from the number and the size values seems to be almost constant regardless of the age of the rabbit.

As described above, the islet cells grow in size and number but the number of the islets decrease with the growth of the animal all the values attaining constant levels between the 210th and the 240th days after birth.

(2) Findings on Langerhans islets of alloxan diabetic F rabbits

(a) α-cells: There is no histological change observable in α-cells and little or no difference in their number from that in normal control rabbits. In size, the α-cells are smaller in F rabbits and the inferiority in size tends to grow as the diabetic generation descends (Fig. 52).

(b) β-cells: There is here also little histological change to speak of, but the number and the size of these cells decrease with marked regularity.

As to the number of β-cells, it is always smaller in F rabbits than in normal

rabbits, becoming smaller and smaller from generation to generation. As shown in the Table, this tendency is quite apparent as early as on the 30th and 40th day after birth and becoming very definite on the 120th day. In P it stands at 31.4 per islet but in F_1, F_2, F_3 and F_4, the number falls gradually from 27.2 to 22.5, 16.0 and 13.3 respectively. The tendency continues thereafter too, the values in P, F_2 and F_3 one year after birth registering 39.0, 24.3 and 22.1 respectively.

The size of β-cells also show the same tendency of decrease. In F rabbits, they are always smaller than in normal rabbits, in the period between 30 and 360 days after birth, generally growing smaller with the descent in genealogy. For example, on the 120th day after birth, the values in P, F_1, F_2, F_3 and F_4 stand at 163, 142, 130, 129 and 104 μ^2 in area respectively (Fig. 52).

In short, we see that hypoplasia is marked in β-cells of the Langerhans islets of F rabbits, and this hypoplasia becomes more strongly marked as the generation goes down in successive diabetic rabbit families.

As given above, while the number of α-cells remains nearly unchanged in F rabbits, the number of β-cells are reduced in them, especially remarkably in F_3 and F_4 rabbits, and this results in the increase of the islets consisting solely of α-cells, which are only rarely observable on sections from normal rabbits, with the descent in generation, so that such all α-cells islets are comparatively frequently found on the sections from F_3 and F_4 rabbits (Fig. 53).

(c) Size of Langerhans islets: As described above, the β-cells constituting the main content of the Langerhans islets in the pancreas become hypoplasied in F rabbits, the α-cells also tending to become smaller. Consequently, it might be inferred that the islets themselves become smaller. The results we obtained, as shown in the Table, revealed that the islets are always smaller in F rabbits than in the control rabbits, becoming smaller from generation to generation, thus: $79\times100\,\mu^2$ in P, $74\times100\,\mu^2$ in F_1, $69\times100\,\mu^2$ in F_2, $63\times100\,\mu^2$ in F_3 and $47\times100\,\mu^2$ in F_4.

(d) Number of Langerhans islets: As the counting of Langerhans islets is a very laborious work, we tested F rabbits of 120 days in age alone for the count. The results showed that in F_1 and F_2 rabbits, the number was not different from that in control rabbits but in F_3 rabbits, we felt it was smaller, though the cases tested of F_3 were too few for definite conclusion. We could test only one case of F_4 and this showed the number of 29 islets. In short, the number of islets in F rabbits down to F_4 is equal to or somewhat smaller than that in control rabbits, but no tendency of increase is ever seen.

(e) Volume occupied by cells in 1 mm^3 of Pancreas: Comparing the results obtained with rabbits of 120 days in age that have been tested for the number of islets, α-cells showed the values of 8.7×, 8.2×, 11.2×, 4.4× and 5.9× (if the number of islets is taken as 29. If the number is 20, the term is reduced to 3.6×) 100,000 μ^3 in the order of P, F_1, F_2, F_3 and F_4. β-cells occupy the volume of 82×, 49×, 36×, 14× and 11× (if the islet number is 20, the term is reduced to 8×) 100,000 μ^3.

Thus, the α-cells show not much difference in total volume in F rabbits

and control rabbits, but the β-cells are decidedly smaller in volume in F rabbits, growing smaller as the generation descends. According to Kwan[72] of our laboratory, who has made a detailed study on rabbits given diabetogenic reagents, the extract of the results concerning the rabbits with severe diabetes but with the β-cells injured least, the rabbits with no diabetes but with the β-cells injured and diminished most severely and with diabetes of medium degree is as shown in Table XIX. As insulin is made from β-cells and the injury to cells other than β is light following administration of diabetogenic reagents, we can get an idea of the approximate extent to which the β-cells are injured in rabbits when pancreatic (β-cell injury) diabetes is induced in them from this Table. Thus, we may assume that the limit of diabetes incidence is laid in the range where the volume of β-cells in 1 mm^3 of pancreas falls between $8.4\times100{,}000\,\mu^3$ and $4.5\times100{,}000\,\mu^3$ and severe diabetes in rabbits is induced when the volume of β-cells is reduced to $4.5\sim1.0\times100{,}000\,\mu^3$ per mm^3 of pancreas.

Now that the volumes of β-cells per mm^3 of pancreas have been computed, as given above, as $14\times100{,}000\,\mu^3$ in F_3 and $11\times100{,}000\,\mu^3$ or $8\times100{,}000\,\mu^3$ in F_4, we may infer that the volume of β-cells in F_4 rabbits on the 120th day after birth comes dangerously near or falls below the limit of diabetogenesis.

Double charge test of sugar

Thus we presume that the reduction of β-cells in F_4 rabbits of 120 days is approaching or has attained the threshold of diabetogenesis. For making this presumption also functionally sure, we made this test, with the following results:

The Staub's effect showed always positive results in normal, F_1 and F_2 rabbits, but of the 5 F_3 rabbits tested 2 cases showed negative and 2 positive, while in 5 F_4 rabbits, 3 had negative and only 1 positive results. According to the study of Sakai *et al.*[74] of our laboratory, when the β-cells are injured and falls below 16 cells per islet following administration of alloxan etc., the Staub's effect in sugar charging test show negative results. In our F_3 and F_4 rabbits, the number of β-cells per islet stands at 16 and 13.3 on the 120th day and at 23 and 18 on the 240th day after birth, and the cells themselves are smaller than the same in normal rabbits. Thus, according to the results obtained by Sakai *et al.*[74] we can easily understand that the Staub's effect in sugar charging tests with these rabbits should come out negative as well as positive. Anyhow, from the results of these tests, our presumption that our F_4 rabbits are in a condition dangerously near to diabetogenesis is amply endorsed.

Cotinued observations of symptoms

We have been led to presume that the condition into which the β-cells in F_4 rabbits have fallen approaches the threshold of induced diabetes from the results of morphological and sugar charging tests. Now, the results of long-continued observations of our F rabbits, including tests of urine sugar and timely tests of blood sugar (usually once a week), are summarized in Table XXI. None of the 13 normal rabbits ever excreted sugar in urine, or showed high blood sugar content and none either of the 4 F_1, 5 F_2, and 5 F_3 cases, except that 2 of the F_1 rabbits temporarily excreted glycosuria of ++ ~ +++ for about one week from the 158th day after birth, showing glycemia of 200~250 mg./dl..

TABLE XIX

Langerhans Islets of Diabetic and Cured Rabbits
(extract of data obtained by Kwan[72])

Animal No.	Treatment	Degree of diabetes mellitus	Urin sugar	No. of cells		Size of cells (μ^2)		No. of islets per 1 mm³ of pancreas	Volume of cells per mm³ of pancreas (10,000 μ^3)	
				α-cells	β-cells	α-cells	β-cells		α-cells	β-cells
1	15th day after administration of 100 mg./kg. of dithizone	Severe	±±±	7.3	3.7	128	118	16.1	35	10
2	300th day after administration of 100 mg./kg. of dithizone	Moderate	+～±±±	5.8	13.5	121	82	17.1	24	45
3	27th day after simultaneous administration of 100 mg./kg. of alloxan and 50 mg./kg. of dithizone	10 days after recovery	−	3.7	9.1	162	167	16.6	49	84

TABLE XX

Double Charge Test of Sugar of Normal and Diabetic Descendant Rabbits

Animal			No. of days after birth	Blood sugar (mg./dl.)							S. E. decision
				bef. adm. ↓	After administration in hrs.						
Generation	Number	Sex			$\frac{1}{2}$	1	$1\frac{1}{2}$ ↓	2	$2\frac{1}{2}$	3	
Normal	12009	♂	254	96	191	92	87	115	108	113	+
	12010	♂	254	109	123	121	104	93	100	83	+
	12011	♀	180	78	147	116	110	117	103	77	+
	12012	♂	180	90	148	126	85	127	120	100	+
	12125	♂	550	80	106	89	76	104	80	96	+
F_1	11123	♂	150	71	121	112	107	114	98	94	+
	11124	♀	210	93	141	119	105	140	111	119	+
F_2	11228	♂	140	89	132	117	93	124	95	72	+
	11229	♀	155	87	124	107	100	120	99	73	+
F_3	11363	♂	545	72	118	112	95	104	103	101	+
	11364	♀	545	83	143	154	140	150	127	104	+
	11385	♂	360	68	105	91	73	104	105	78	±
	11368	♂	545	75	102	104	82	108	107	73	−
	11383	♂	360	73	123	111	96	128	93	78	−
F_4	11426	♀	190	74	92	110	83	102	92	73	+
	11423	♂	190	74	110	96	83	110	110	85	±
	11405	♂	320	81	107	105	98	117	111	96	−
	11408	♂	320	97	102	110	93	115	127	95	−
	11415	♂	213	77	98	95	78	114	82	88	−

After fasting for 24 hours, 0.5 g./kg. each of 10% water solution of glucose injected subcutaneously at interval of 90 minutes. ↓ marks the time of glucose injection.

18 of the 18 cases, however, of the F_4 and 7 of the 7 F_5 rabbits tested became all diabetic. The diabetic conditions or the sugar contents of urine and blood in suspected cases of diabetes were as shown in Table XXII. That the sugar indicated in these results was glucose has been ascertained by frequent fermentation tests and osazone generation reaction tests made in combination with Benedict's tests. 11417, 11425 and 11428, in particular the latter two, showed symptoms of severe diabetes continuously from the first attack downwards and 11424 and 11427 were in similar conditions till they died from vehement diarrhea 10 and 41 days respectively after the incidence. The other cases are going on repeating excreting glycosuria for several days intermittently, showing high blood sugar content during these periods.

TABLE XXI

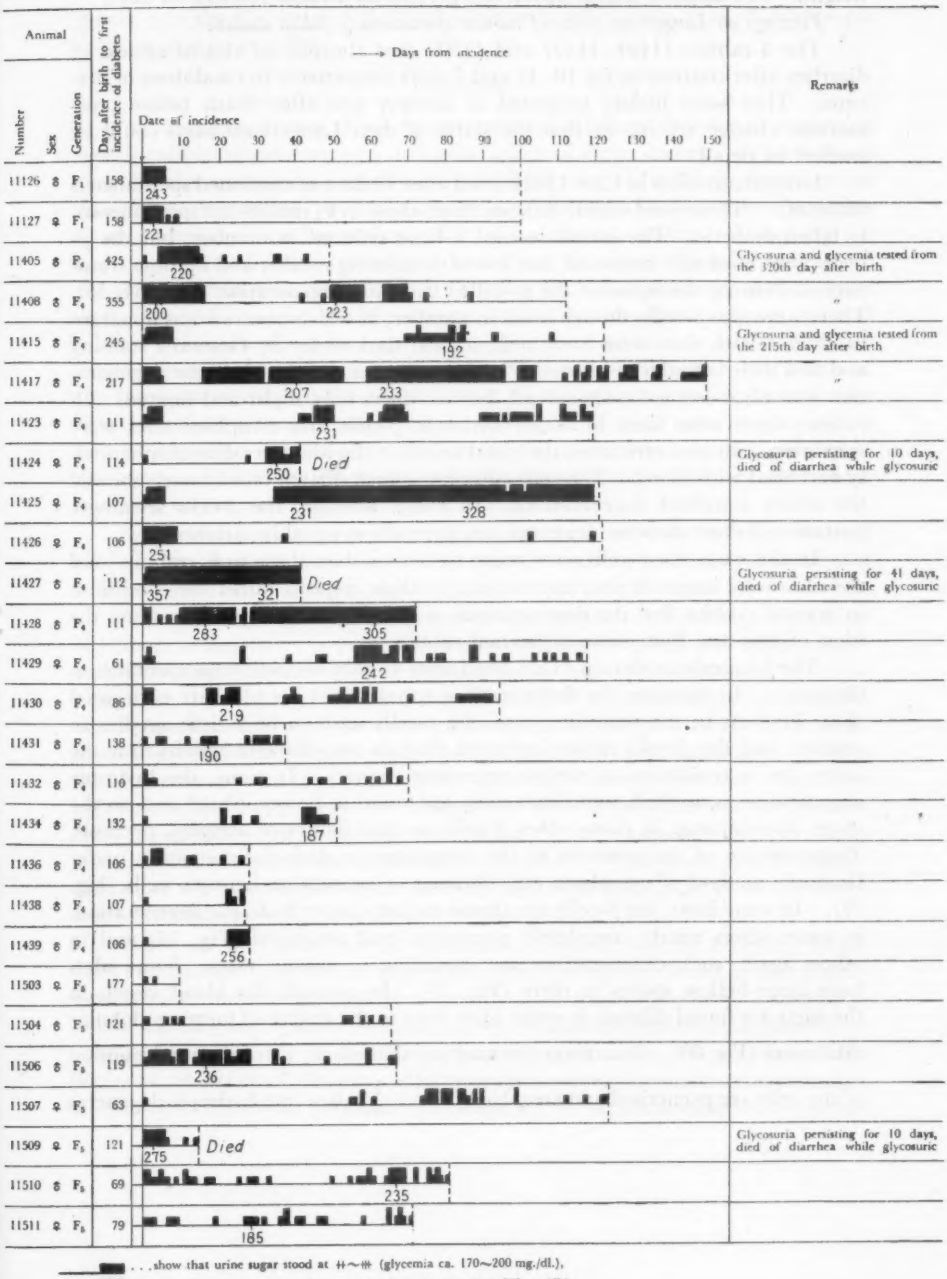
Long Continued Observation of Progress in Normal and Diabetic Descendant Rabbits that Lived for More than 120 Days after Birth (March 31, 1954)

Animal				Date of examination after birth	No. of rabbits	Occurrence of diadetes	Total no. of incidence of diabetes	Incidence of diabetes
Generation		Number	Sex					
Normal rabbits	P	1001	♂	42～516	6	−	0	
		1002	♀	42～516		−		
		1003	♂	42～516		−		
		1004	♂	42～516		−		
		1005	♀	50～502		−		
		1006	♀	50～502		−		
	F_1	12002	♀	37～736	5	−	0	
		12009	♂	37～736		−		
		12010	♂	40～710		−		
		12011	♀	40～710		−		
		12012	♂	40～710		−		
	F_2	12125	♂	70～245	2	−	0	
		12128	♀	75～250		−		
Rabbits born of ancestors successively made diabetic every generation	F_1	11123	♂	142～243	4	−	2…?*	For about 7 days §
		11124	♀	142～243		−		
		11126	♂	112～213		+…?§		For several days §§
		11127	♀	112～213		+…?§§		
	F_2	11228	♂	43～179	5	−	0	
		11229	♀	43～179		−		
		11230	♂	43～179		−		
		11231	♂	43～179		−		
		11232	♀	43～179		−		
	F_3	11363	♂	340～684	5	−	0	
		11364	♀	340～684		−		
		11368	♂	341～685		−		
		11383	♂	342～686		−		
		11385	♂	342～686		−		
	F_4	11405	♂	320～478	18	+	18	Intermittently
		11408	♂	320～478		+		〃
		11415	♂	215～367		+		〃
		11417	♂	215～367		+		Long persisting
		11423	♂	78～226		+		Intermittently
		11424	♀	78～146		+		Died on 146th day,‡
		11425	♀	78～226		+		Long persisting
		11426	♀	78～226		+		Intermittently
		11427	♀	61～151		+		Died on 151st day,‡
		11428	♂	61～171		+		Long persisting
		11429	♀	34～183		+		Intermittently
		11430	♂	34～183		+		〃
		11431	♂	34～183		+		〃
		11432	♂	34～183		+		〃
		11434	♂	34～183		+		〃
		11436	♂	41～122		+		〃
		11438	♂	41～122		+		〃
		11439	♂	41～122		+		〃
	F_5	11503	♀	34～185	7	+	7	〃
		11504	♂	34～185		+		〃
		11506	♂	34～185		+		〃
		11507	♀	34～185		+		〃
		11509	♀	34～134		+		Died on 134th day,‡
		11510	♂	34～151		+		Intermittently
		11511	♀	34～151		+		〃

Feed: Bean cake refuse, oat (barley), sweet potato, carrots, vegetables and grass mixed and freely fed. * These rabbits were born of a male with glycosuria persisting for 664 days and a female with glycosuria for 194 days and very severely diabetic. Incidence was assumed when blood sugar of more than about 200 mg./dl., and glycosuria stronger than sign ## were observed. ‡ Long persisted.

TABLE XXII

Glycosuria and Glycemia Observed in Rabbits upon Spontaneous Occurrence of Diabetes (March 31, 1954)


Animal
Number
Sex
Generation
Day after birth to first incidence of diabetes
Days from incidence
Date of incidence
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Remarks
11126 ♂ F1 158 243
11127 ♀ F1 158 221
11405 ♂ F4 425 220 Glycosuria and glycemia tested from the 320th day after birth
11408 ♂ F4 355 200 223 "
11415 ♂ F4 245 192 Glycosuria and glycemia tested from the 215th day after birth
11417 ♂ F4 217 207 233 "
11423 ♂ F4 111 231
11424 ♀ F4 114 250 Died Glycosuria persisting for 10 days, died of diarrhea while glycosuric
11425 ♀ F4 107 231 328
11426 ♀ F4 106 251
11427 ♂ F4 112 357 321 Died Glycosuria persisting for 41 days, died of diarrhea while glycosuric
11428 ♂ F4 111 283 305
11429 ♀ F4 61 242
11430 ♂ F4 86 219
11431 ♂ F4 138 190
11432 ♂ F4 110
11434 ♂ F4 132 187
11436 ♂ F4 106
11438 ♂ F4 107
11439 ♂ F4 106 256
11503 ♀ F5 177
11504 ♂ F5 121
11506 ♂ F5 119 236
11507 ♀ F5 63
11509 ♀ F5 121 275 Died Glycosuria persisting for 10 days, died of diarrhea while glycosuric
11510 ♂ F5 69 235
11511 ♀ F5 79 185

. . . show that urine sugar stood at ++~+++ (glycemia ca. 170~200 mg./dl.),

. . . show that urine sugar stood at +++~++++ (glycemia more than 200 mg./dl.)

and . . . show present status. The figures in the lower line shows blood sugar (mg./dl.).

We have pointed out above that the F_4 and F_5 rabbits are generally somewhat faster in growth, and especially the severely diabetic rabbits show avaricious appetite during the 50~150th days after birth and grow at a rash pace in bodyweight. The diabetes begins within this period, on around 100~120th days.

Findings on Langerhans islets of rabbits spontaneously fallen diabetic

The 3 rabbits 11424, 11427 and 11509 died abruptly of violent attacks of diarrhea after continuing for 10, 41 and 7 days respectively in conditions of diabetes. They were luckily subjected to autopsy just after death before postmortem change set in, so that the status of their Langerhans islets could be studied in detail.

Langerhans islets in Case 11424 (died after 10 days of continued spontaneous diabetes) : These were widely different from those in F_4 rabbits not spontaneously fallen diabetic. The α-cells seemed a little reduced in number, but the β--cells were markedly increased, not few of them being swollen and become transparent, showing the figure of the so-called hydropic degeneration (Figs. 54, 55). There were also β-cells, though small in number, in which pycnosis was apparent in their nuclei, that were atrophied, stained dark-violet by Gomori's staining and had their boundaries blurred (Fig. 54). In some of the islets, the degeneration was advanced and wide-spread, but in others only slight and limited. Of course, there were islets in which hydropic β-cells and atrophied ones were coexisting. In very rare cases, the blood vessels in the islet were dilated spherically and filled with blood. The cells adjacent to such dilated blood vessels showed the above described degenerations. In rough average, the β-cells seemingly normal and those showing degenerations were about equal in number.

In this case, the β-cells were more numerous than those in F_4 rabbits and the islets were larger in size, approaching in these respects rather the conditions in normal rabbits, but the degenerations in the β-cells clearly differentiate the islets of this case from those of normal rabbits.

The Langerhans islets in 11427 (died after 41 days of continuous spontaneous diabetes) : In this case, the degenerations appearing in the islets are variegated (Fig. 56). As in the preceding case, the α-cells seem to be a little smaller in number and the β-cells rather increased than in non-diabetic rabbits, but the latter are in conditions of various regressive changes. In some, the hydropic degeneration is marked, sometimes these are found to be vecuolated and on the verge of collapsing, in many other β-cells we find figures of atrophy, pycnosis, disappearance of the granules in the cytoplasm or dark-violet staining under Gomori's method of cytoplasm and blurring of boundaries between cells (Fig. 57). In some islets, the β-cells are almost entirely under hydropic degeneration, in some others nearly completely pycnotised and atrophied (Fig. 58) and in others again, such degenerations are coexisting in various rates. Some islets have large hollow spaces in them (Fig. 59). In general, the blood vessels in the islets are found dilated, in some islets even to the degree of forming globular dilatations (Fig. 60). In average, we may say that about $\frac{1}{2}$ of the total number of the cells are pycnotised and atrophied, about $\frac{1}{4}$ fallen into hydropic degenera-

tion and the remaining $\frac{1}{4}$ in normal healthy condition.

Langerhans islets of Case 11509 (died after 7 days of spontaneous diabetes): Generally similar to those in 11427 but not so advanced in degeneration. The β-cells here show pycnosis and atrophy in considerably many cases, but hydropic degeneration in very few.

Discussion

As described above, if we repeat inducing alloxan diabetes in male and female rabbits at random for 4 or 5 generations of direct descent, we have found that diabetes occurs spontaneously in F_4 and F_5 rabbits. It is clearly demonstrated that this spontaneously induced diabetes is pancreatic in nature from the results of examination of the Langerhans islets of the diabetic rabbits. Observing the diabetogenic conditions of the rabbits from the point of sugar in urine and blood, the sugar content is found to be little different from that of normal rabbits down to F_3, but in F_4 and F_5, the blood sugar content rises and glycosuria is excreted all of a sudden, and we are impressed as if a new variation of rabbits has suddenly appeared, but when we examine the process of change in the β-cells of the Langerhans islets in the rabbits down to the generation of F_4, we can see that the variation is not abrupt, but the change in the islets have been amplified step by step from generation to generation, till the rabbits become incapable of producing sufficient insulin to assure disposition of carbohydrates in their body, resulting in the appearance of diabetic syndromes.

Such results as we have described in the above have never been reported to date, but as there have been a few reports touching on some points related with our observation, we will speak a word on such report.

As to human subjects, there have been some reports on the Langerhans islets of fetus or infants born from diabetic mothers since Dubreuil and Anderodias[76] and Gray and Feemster[77] have published their reports, as enumerated in the reports by Potter *et all.*,[78] Miller[79] and Cardell.[80] According to these reports, the Langerhans islets in the fetus or newborn infants from diabetic mothers show hyperplasia and hypertrophy, and many researchers deem these changes as compensatory for diabetes. More recently, however, it was found that hyperplasia and hypertrophy are observed in islets of fetus and newborn infants from prediabetic or non-diabetic mothers also,[78,79,80,81] especially comparatively often in islets of the case of erythroblastosis foetalis[78,79,80], and also that the hyperplasia and the hypertrophy in the islets in the infants are not proportional to the degree of diabetes of the mothers.[78]

There are also some animal experiments reported on the subject. Friedgood and Miller[82] say that no hyperplasia or hypertrophy is found in the Langerhans islets of the youngs born from pregnant rats made diabetic by administration of alloxan, and Ibaraki[83] of our laboratory also has reported that there is no marked change found in the Langerhans islets in youngs rabbits born from pregnant rabbits made diabetic. Hultquist,[84] however, has made detailed studies on the offsprings from pregnant rats subjected to depancreatization at several

stages. Insulin was administered to most of the mother rats, but many of the youngs were of giant growth, showing frank hyperplasia of most of their endocrine glands and more or less enlargement of their thymus and Langerhans islets. In normal-sized youngs also the Langerhans islets showed some increase in their volume. These are reports on the cases where diabetes was induced in pregnant animals, but there are some reports also on the cases where diabetic female animals have become pregnant and were delivered of youngs. Gordon[85] has reported that increase of islet tissues was found in offsprings from depancreatized bitches, and Bailey and Bailey[25] that the Langerhans islets, as in the cases of human subjects cited above, were hypertrophied in the offsprings from alloxan-diabetic rabbits. But since the original report by Bailey and Bailey is not made public, the accuracy of this citation may be deemed as subject to doubt. As reported in the preceding chapters, we have studied normal rabbits and F rabbits from alloxan-diabetic parents on both sides for 30 days~1 year in comparison, and have observed that the Langerhans islets were always smaller in F rabbits and that this diminution in islet size was due to the hypoplasia of β-cells, a result not in agreement with the above-mentioned cases, especially with the observed cases in human subjects. Moreover, the degree of change in the Langerhans islets, to our interest, we found to increase gradually from generation to generation. The results of our studies on F rabbits may be deemed as of a quite different category from three obtained by the predecessors cited above in that (1) our results were obtained not from new born youngs, but from rabbits at least 30 days old and (2) our rabbits were not bred from diabetic mothers only, but from males and females both made diabetic and paired among themselves after the lapse of some time after the diabetization. But seeing that the reports on fetus and new-born infants from diabetic mothers give discrepant results as cited above, we believe further detailed studies are required on this subject in the future.

In the next place, we may point to some studies on the heredity and other points related with diabetic obese mice,[86,87,88,89,90] but there is a wide difference between these and our studies. These diabetic obese mice were all discovered by chance, and there have been no experiment for generating such mice purposely. Moreover, it seems that the diabetes in these mice is not pancreatic in nature.

Thus, the above studies of ours are of a nature not to be seen in the past studies on diabetes, and there have been neither many studies concerning the other phases, at least with higher animals as subject, reported to date. We will here under look over the few studies on such subjects in comparison with our studies.

Of old, Weismann[91] has reported that even when the tails of mice were cut short for about 20 successive generations, the tails became not a bit shorter. This seems in complete disagreement with the results we have obtained, but there is apparently a great difference in the experiments by Weismann and by us. We cannot believe that in a mouse whose tail was cut in his experiment there occurred any perceptible change in the metabolism in its somatic substances. In our experiments, the rabbits were given alloxan, followed by occurrence of diabetes causing a vehement change in metabolism. Such a difference was

apparently the factor that led to the discrepancy in the results by Weismann and this laboratory.

Rowntree, Clark and Hanson[92] gave extract of thymus to rats, and repeating the experiment for four generations, have reported that the growth is expedited in the descendants, the more as the generation goes down. Einhorn and Rowntree[93] have repeated the experiment of excising the thymus of parent rats for two generations and reported that the growth is gradually retarded in the descendants. It is of interest that these experiments have many points of similarity with the ours. It is regrettable, however, that in their reports nothing is said upon the question of how the expedited or retarded growth had been brought about. We know that there are many factors, both internal and external, that may influence bodily growth, and we regret that there is no explanation as to whether the changed pace in the growth of F rats was occasioned by a change, morphological or functional, in the thymus or any other organ of the F rats.

Guyer and Smith[94] have observed that when a rabbit's lens tissue is given intraperitoneally to a fowl and the serum taken from this fowl injected intravenously to a pregnant rabbit, though no change comes on the eyes of this mother rabbit, various deformities, especially cataract, appear in the eyes of the youngs and are inherited by their descendants. The fact that a change in the heredity of a certain bodily part is brought by a specific external operation is worthy of special mention in itself, but as to how this change has been occasioned, the authors stated only that it was uncertain whether the result should be reckoned as an inheritance of a somatic modification, that is, the anomaly in the eyes of the embryos have caused a change in the germ cells secondarily or a change occurs simultaneously in both the lens and the germ cells. At present, it seems that the latter explanation of a mutation of germ cells by the immunization serum is generally accepted. The results of these experiments are probably worthy of consideration in studying the mechanism whereby the change in Langerhans islets is brought about in our experiments, but their experiments are not of the same kind with ours. In the experiments by Guyer and Smith, pregnant mother rabbits were operated upon and the change was occasioned in the embryos in the maternal body. Moreover, in their case, a perceptible modification was effected in one generation. In our experiments, the operation was applied to parent rabbits on both sides, followed by a permanent change in the parents, and then they were paired. Further, the change in the islets in F rabbits was only light in the first generation, being fortified in subsequent generations, to cause appearance of frank symptoms first in the F_4 and F_5 rabbits.

Kusagawa[95] as well as Pagenstecher[95] and v. Szily[95] have found cataracts in the eyes of chickens hatched from eggs to which naphthalin or similarities was applied or laid by hen to which naphthalin had been administered. Kusagawa has observed that this deformity is inherited by descendants. In this case, as in the Guyer and Smith's study, an inherited modification was brought about in one generation.

Strong[96] had subcutaneously given methylcholanthrene to mice of mixed

breed of three strains (NH descent) and gone on selecting youngs showing local tumor resistance, till he obtained mice apt to grow cancer in the pyloric part of the stomach. After continuing such a selection and administration of methylcholanthrene for generations, he finally arrived at mice that had spontaneous stomach cancer without being subjected to the injection, and thus had obtained the interesting observation that this spontaneous cancer incidence is inherited. He says that in his case, the methylcholanthrene used in his experiments has acted both in changing the gastric mucosa into cacer and in causing germinal mutation in the mice. He states, however, that the possibility that this mutation is a spontaneous one and methylcholanthrene has only acted favorably for the happening of the mutation is not excluded. In this study, the mice in which cancer was induced under administration of methylcholanthrene were selected for many generations till a descent in which stomach cancer spontaneously occurs was obtained. In our experiments, however, we have never made an attempt at selecting a descent with hypoplasia in β-cells, and this is a great difference between the two sets of experiments.

In short, there have been indeed some studies that have arrived to results analogous to ours, some among them arriving at results very little different from ours, but we have heard nothing about a conclusion as to how such results have been obtained. This is presumably due to the fact that the solution of this problem is directly connected with the solution of the yet unsettled difficult question long discussed in biological circles, the question whether a change effected by an external factor on somatic cells exercises any influence on germ cells or not.

We have indeed obtained the results by our experiment as described above and now we must face the question how these results were arrived at. And in our case, the question presents many points of difficulty as in all such similar experiments. We and our colleagues in our laboratory are coping with this question in all earnest. That is, we are giving all our efforts for elucidating what, when, where and how the factor has acted upon in causing the hypoplasia of β-cells or occurrence of diabetes, what is the nature of this factor, and is it inheritable or not, and expect to make a separate report thereon in a near future.

Summary

Male and female rabbits were made alloxan-diabetic, paired among themselves a certain time after the diabetization and the youngs begotten then were named rabbits F_1. These F_1 rabbits were similarly made diabetic upon attaining maturity, paired and made to breed. Such a procedure was repeated for five generations and the resulting $F_1 \sim F_5$ rabbits were made the subject of our study. The results were as follows:

1. In increase of bodyweight after birth, little difference was found between the normal and the $F_1 \sim F_5$ rabbits, except that the F_3 rabbits were slightly inferior in this respect and the F_4 and F_5 rabbits somewhat better, especially around $60 \sim 120$ days after birth.
2. The β-cells in the Langerhans islets in the pancreas of F rabbits were

found suffering from hypoplasia. It was evidenced by the decrease both in number and in size of β-cells per islet seen on the sections, observable in all ages from 30 days downwards, and in fact this reduction of cells progressed step by step from generation to the next. For example, the number of β-cells per islet was 31.4, 27.2, 22.5, 16.0 and 13.3 and their size 163, 142, 130, 129 and 104 μ^2 respectively in P, F_1, F_2, F_3 and F_4 rabbits 120 days after birth.

3. The α-cells in F rabbits showed the tendency of slowly decreasing in size from generation to generation (at 120 days after birth, the respective areas showed the values of 148, 129, 126, 120, 111 μ^2 in P and $F_1 \sim F_4$ rabbits), but their number did not change appreciably.

4. The size of Langerhans islets was always smaller in F rabbits than in normal rabbits from 30 days after birth downwards, and this reduction in size progressed from generation to generation. For example, the mean area of the islets at 120 days after birth was 79.3 $\times$, 74.2 $\times$, 68.6 $\times$, 62.8 $\times$ and 47.1 $\times$ 100 μ^2 respectively in P and $F_1 \sim F_4$ rabbits.

We found no marked change in the number of Langerhans islets.

5. Based upon the assumption that the Langerhans islets are spherical in shape and utilizing the data of measurements of the size and the number of the islet cells and the number of islets in rabbits 120 days of age, we made a tentative calculation of the volume occupied by the cells in 1 mm^3 of pancreas, and have found that the total volume of β-cells per mm^3 of pancreas decrease by leaps and bounds. Thus, the value stands at 82 $\times$ 100,000 μ^3 normal rabbits but drops to 49 $\times$ 100,000 μ^3 in F_1, to 36 $\times$ in F_2, to 14 $\times$ in F_3 and to 11 (or 8) $\times$ 100,000 μ^3 in F_4 rabbits. In F_4 rabbits, the volume drops to about $\frac{1}{8} \sim \frac{1}{10}$ of normal rabbits, and this value is presumed to be dangerously near or approximately equal to the threshold value of incidence of diabetes caused by injury in β-cells. The total volume of α-cells showed slight reduction but much less remarkable than that of β-cells.

6. The double charge sugar test conducted on normal and F rabbits showed that while all cases of normal, F_1 and F_2 rabbits resulted positively in Staub's effect, some F_3 and the majority of F_4 rabbits showed negative results.

7. By a continued observation of the rabbits for more than 120 days after birth, we found no spontaneous occurrence of diabetes (diabetes without giving diabetogenic reagents or taking any external means) in F_1, F_2 and F_3 rabbits, not to speak of the normal rabbits. In F_4 and F_5 rabbits we found sure signs of spontaneous occurrence of diabetes. 18 of the 18 F_4 rabbits and 7 of the 7 F_5 rabbits fell to diabetes, between 61 to 425 days after birth. Three among the diabetic cases were of permanent nature (continuing for 73, 122 and 150 days respectively), 3 others died of diarrhea on 7th, 10th, and 41st days of continued and severe diabetes, and the remaining cases are repeating excretion of glycosuria for several days at intervals. The cases of severe diabetes showed surprising appetite and remarkable growth in bodyweight in the 50th $\sim$ 150th days after birth.

8. An examination of Langerhans islets in severe spontaneous diabetes

cases showed that, in one case of diabetes continued for a short period, the α-cells tended to decrease slightly and the β-cells to increase and also that the β-cells were in many cases frankly subject to hydropic degeneration, the remaining normal, and in another case considerably many of the cells were atrophied and pycnotic. In the cases where the diabetes has continued somewhat longer, the normal-looking β-cells were very few, most of the β-cells being pycnotised or atrophied, intermingled by those showing hydropic degeneration. This observation led us clearly to identify the spontaneous diabetes occurring in the F rabbits as pancreatic (or insular) diabetes.

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Fig. 2. Fig. 3.

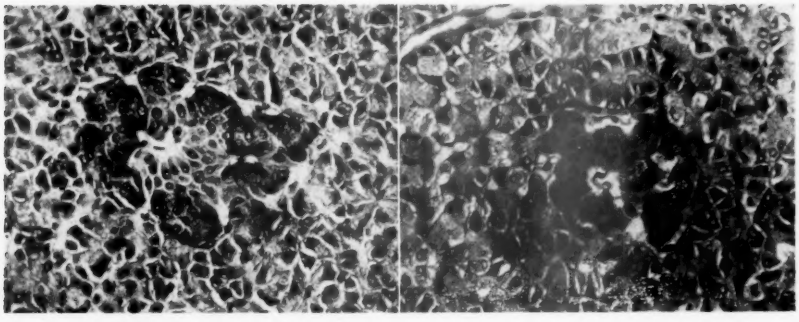

Fig. 2. Langerhans islet of a normal rabbit (Gomori's staining). β-cells constitute the chief component, α-cells surrounding them to form the periphery of the islet.

α-cells · · · dark (red) β-cells · · · light (blue)

Fig. 3. Langerhans islet of a rabbit, 30 minutes after intravenous injection of 40 mg./kg. of 8-hydroxyquinaldine (Gmori's staining). Boundaries of β-cells are obscure and seen several pycnotized nucleus in them. α-cells not markedly changed.

Fig. 4. Fig. 5.

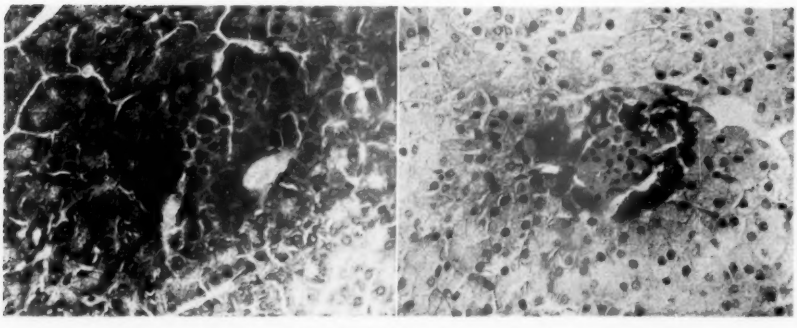

Fig. 4. Langerhans islet of a rabbit, 12 hours after intravenous injection of 40 mg./kg. of 8-hydroxyquinaldine (Gomori's staining). Nuclei of β-cells markedly pycnotized, a few β-cells necrotic and already collapsed. No marked change in α-cells.

Fig. 5. Langerhans islet of a rabbit, 24 hours after intravenous injection of 40 mg./kg. of 8-hydroxyquinaldine (Gomori's staining). The central part greatly necrotic, showing many debris of nuclei, α-cells in the periphery not markedly changed.

Fig. 6. Fig. 7.

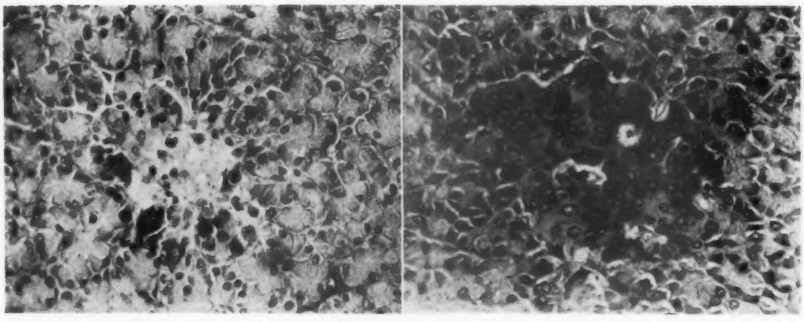

Fig. 6. Langerhans islet of a rabbit, 48 hours after intravenous injection of 40 mg./kg. of 8-hydroxyquinaldine (Gomori's staining). The central part strongly necrotic and turned into a disorganized homogenous mass, only a few pycnotized nuclei being observed therein. Almost no β-cells. Little changed α-cells observed at the periphery.

Fig. 7. Langerhans islet of a rabbit, 5 days after intravenous injection of 40 mg./kg. of 8-hydroxyquinaldine (Gomori's staining). β-cells reduced in number, some of them hypertrophied. No marked change in α-cells.

Fig. 8. Fig. 9.

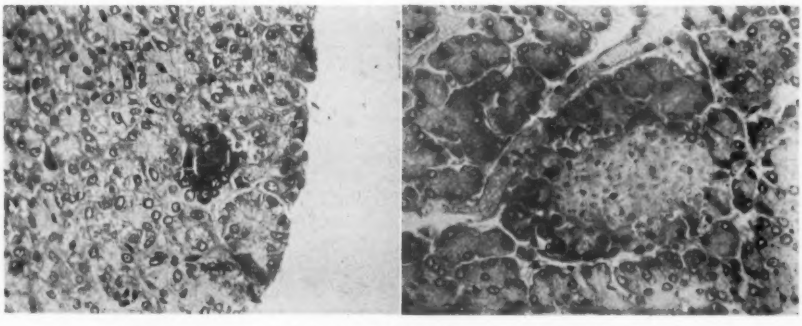

Fig. 8. Langerhans islet of a rabbit, 120 days after intravenous injection of 10 mg./kg. of 8-hydroxyquinaldine (Gomori's staining). Islet markedly dwarfed, consisting only of seemingly somewhat atrophied α-cells and no β-cells.

Fig. 9. Langerhans islet of a rabbit, 12 hours after intravenous injection of 25 mg./kg. of 5-(phenylazo)-8-hydroxyquinoline (Gomori's staining). Homogenous necrotic mass formed at the center, with several nuclear debris in it. No marked change in the α-cells in the periphery.

Fig. 10. Fig. 11.

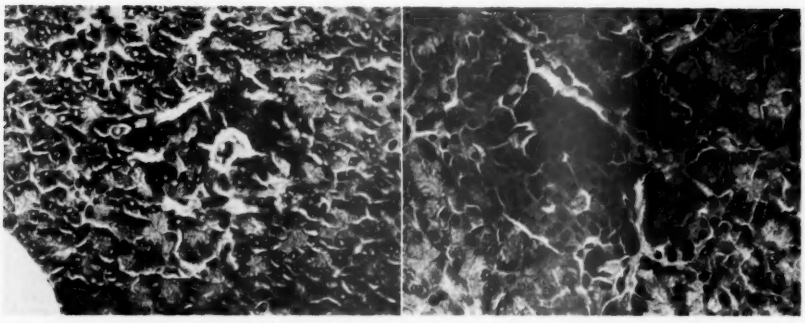

Fig. 10. Langerhans islet of a rabbit, 19 days after intravenous injection of 20 mg./kg. of 5-(phenylazo)-8-hydroxyquinoline (Gomori's staining). Islet almost entirely occupied by α-cells, only a few β-cells intermingling.

Fig. 11. Langerhans islet of a rabbit, 30 minutes after intravenous injection of 10 mg./kg. of 5-(phenylazo-)8-hydroxyquinaldine (Gomori's staining). Almost all of nucleus in β-cells show frank pycnosis. No marked change in α-cells.

Fig. 12. Fig. 13.

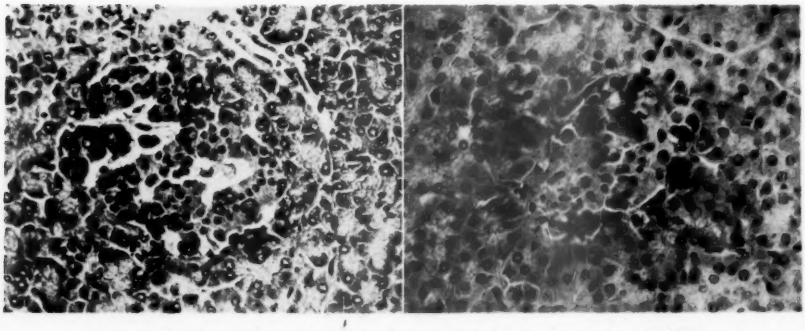

Fig. 12. Langerhans islet of a rabbit, 12 hours after intravenous injection of 20 mg./kg. of 5-(phenylazo)-8-hydroxyquinaldine (Gomori's staining). β-cells degranulated, atrophied and collapsing, with their nuclei pycnotized. No marked change in α-cells.

Fig. 13. Langerhans islet of a rabbit, 24 hours after intravenous injection of 10 mg./kg. of 5-(phenylazo)-8-hydroxyquinaldine (Gomori's staining). The β-cells at the center turned into a homogenous necrotic mass, containing nuclear debris. Strong nuclear debris in remaining β-cells. No marked change in α-cells.

Fig. 14. Fig. 15.

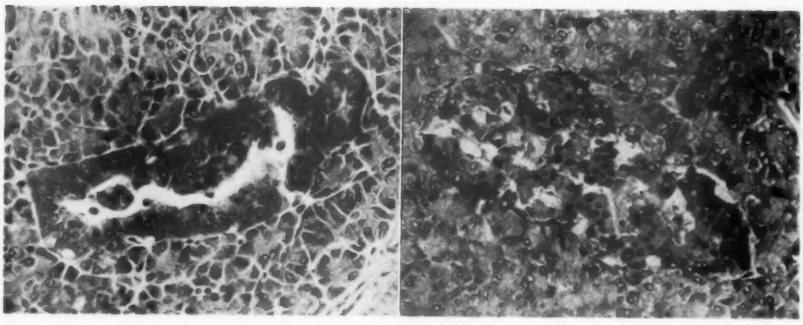

Fig. 14. Langerhans islet of a rabbit, 48 hours after intravenous injection of 10 mg./kg. of 5-(phenylazo)-8-hydroxyquinaldine (Gomori's staining). Islet nearly made up of *a*-cells only, an extensive gap formed at the center, seemingly by collapse and elimination of β-cells.

Fig. 15. Langerhans islet of a rabbit, 4 hours after intravenous injection of 40 mg./kg. of 2,4-dimethyl-8-hydroxyquinoline (Gomori's staining). β-cells show frank pycnosis in nucleus and seem ready to collapse, while a part has formed homogenous necrotic mass. No marked change in α-cells.

Fig. 16. Fig. 17.

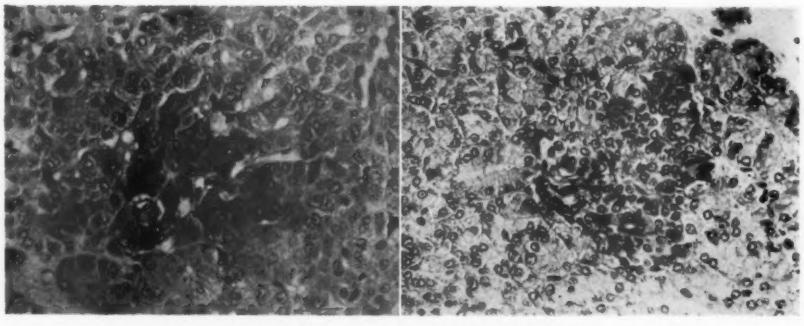

Fig. 16. Langerhans islet of a rabbit, 15 days after intravenous injection of 40 mg./kg. of 2,4-dimethyl-8-hydroxyquinoline (Gomori's staining). Islet composed only of α-cells and no β-cells in sight.

Fig. 17. Langerhans islet of a rabbit, 179 days after intravenous injection of 20 mg./kg. of 5-(*p*-tolylazo)-8-hydroxyquinoline (Gomori's staining). Islet consisting almost entirely of unchanged α-cells, with few β-cells with reduced granules or with advanced hydropic degeneration mingled among them.

Fig. 18. Fig. 19.

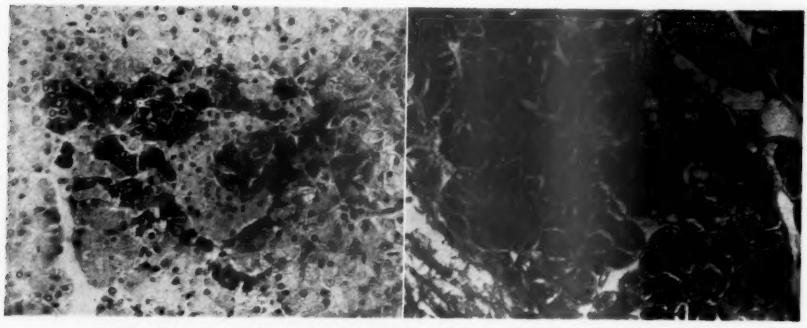

Fig. 18. Langerhans islet of a rabbit, 12 hours after intravenous injection of 40 mg./kg. of 5-(*o*-tolylazo)-8-hydroxyquinoline (Gomori's staining). Extensive homogenous necrotic mass surrounded by nearly normal α-cells. Sporadic nuclear debris in the mass.

Fig. 19. Langerhans islet of a rabbit, 30 minutes after intravenous injection of 40 mg./kg. of 5-(*p*-hydroxyphenylazo)-8-hydroxyquinoline (Gomori's staining). β-cells turned into collapse and showing severe nuclear pycnosis. α-cells not markedly changed.

Fig. 20. Fig. 21.

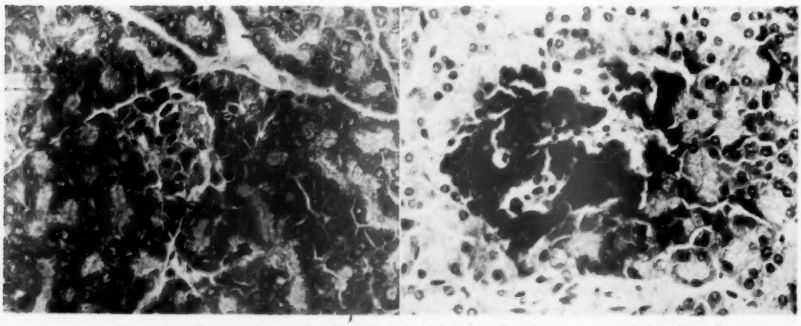

Fig. 20. Langerhans islet of a rabbit, 2 hours after intravenous injection of 50 mg./kg. of 5-(*p*-hydroxyphenylazo)-8-hydroxyquinoline (Gomori's staining). Nuclei of β-cells markedly pycnotized. At some places β-cells are necrotic and have become a homogenous mass. α-cells not markedly changed.

Fig. 21. Langerhans islet of a rabbit, 4 hours after intravenous injection of 40 mg./kg. of 5-(*p*-hydroxyphenylazo)-8-hydroxyquinoline (Gomori's staining). Islet almost entirely made up of α-cells. Most of the β-cells had collapsed and been eliminated. Some remaining nuclear debris seen scattered.

Fig. 22. Fig. 23.

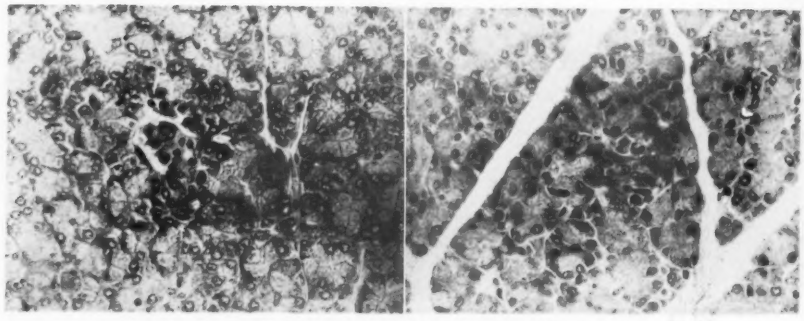

Fig. 22. Langerhans islet of a rabbit, 12 hours after intravenous injection of 40 mg./kg. of 5-(*p*-hydroxyphenylazo)-8-hydroxyquinoline (Gomori's staining). Islet almost entirely composed of α-cells, a few β-cells with pycnotized nuclei scattered among them.

Fig. 23. Langerhans islet of a rabbit, 24 hours after intravenous injection of 40 mg./kg. of 5-(*p*-hydroxyphenylazo)-8-hydroxyquinoline (Gomori's staining). Almost all of β-cells had been absorbed and been eliminated already. Islet almost entirely made up of α-cells without marked change.

Fig. 24. Fig. 25.

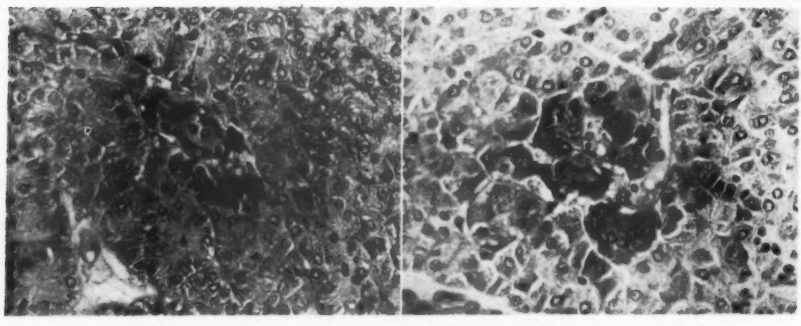

Fig. 24. Langerhans islet of a rabbit, 5 days after intravenous injection of 50 mg./kg. of 5-(*p*-hydroxyphenylazo)-8-hyrdoxyquinoline (Gomori's staining). Islet almost entirely made up of α-cells, but a few β-cells remained in it.

Fig. 25. Langerhans islet of a rabbit, 48 hours after intravenous injection of 40 mg./kg. of 5-(*p*-hydroxyphenylazo)-8-hydroxyquinaldine (Gomori's staining). Islet mainly composed of slightly hypertrophied α-cells. Several cells showing advanced nuclear pycnosis.

Fig. 26. Fig. 27.

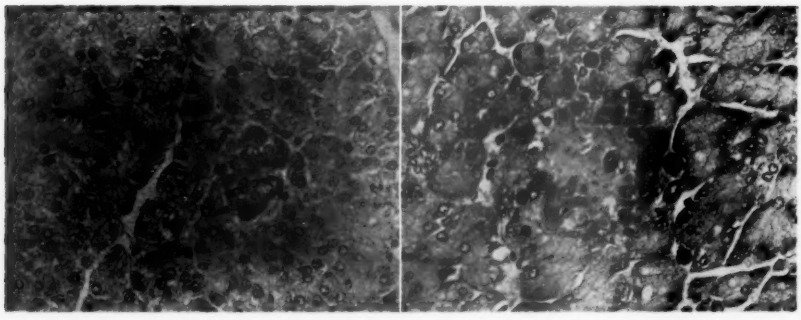

Fig. 26. Langerhans islet of a rabbit, 48 hours after intravenous injection of 70 mg./kg. of 5-(*p*-dimethylaminophenylazo)-8-hydroxyquinoline (Gomori's staining). Islet nearly made up of α-cells only.

Fig. 27. Langerhans islet of a rabbit, 15 hours after intravenous injection of 30 mg./kg. of 5-(*p*-dimethylaminophenylazo)-8-hydroxyquinaldine (Gomori's staining). Homogenous necrotic mass occupying the central part and α-cells without marked changed surrounding it.

Fig. 28. Fig. 29.

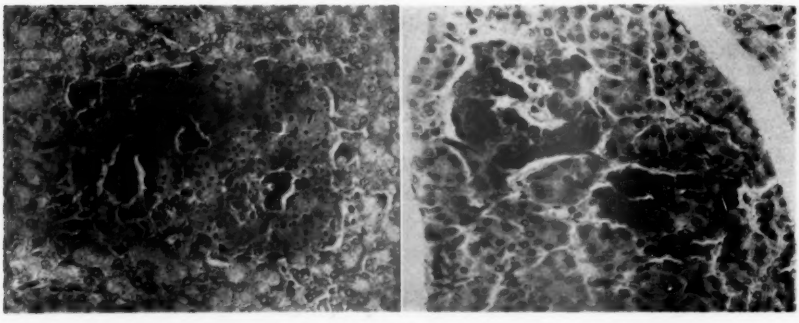

Fig. 28. Langerhans islet of a rabbit, 40 hours after intravenous injection of 50 mg./kg. of 5-(*p*-acetaminophenylazo)-8-hydroxyquinoline (Gomori's staining). Islet largely occupied by β-cells in extensive necrosis, with heavily pycnotized nuclei. A part already collapsed. No marked change in α-cells.

Fig. 29. Langerhans islet of a rabbit, 72 hours after intravenous injection of 10 mg./kg. of 5-(*p*-aminophenylazo)-8-hydroxyquinoline (Gomori's staining). 2 islets both almost made up of α-cells, only a few β-cells seen at the center and some peripheral parts.

Fig. 30. Fig. 31.

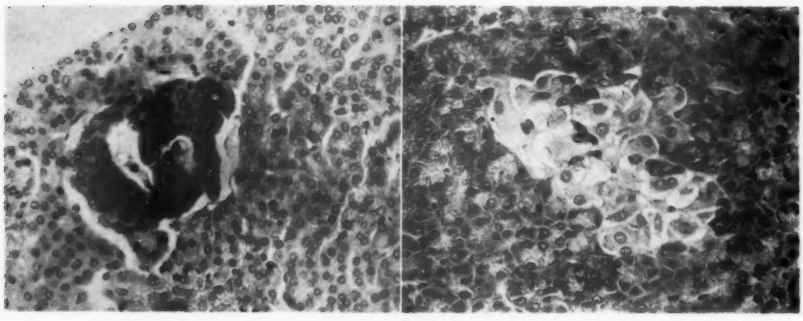

Fig. 30. Langerhans islet of a rabbit, 72 hours after intravenous injection of 5 mg./kg. of 5-(*p*-acetaminophenylazo)-8-hydroxyquinaldine (Gomori's staining). Islet composed of not much changed α-cells and of a few β-cells and shows 2 gaps at the center, seemingly formed by collapse and elimination of β-cells.

Fig. 31. Langerhans islet of a rabbit, 33 days after incidence of diabetes with intravenous injection of nicotine (Gomori's staining). β-cells markedly swollen, many of them with large vacuoled or reticular substance in it, some without nucleus and some dissolved. In short, a figure of hydropic degeneration is presented.

Fig. 32. Fig. 33.

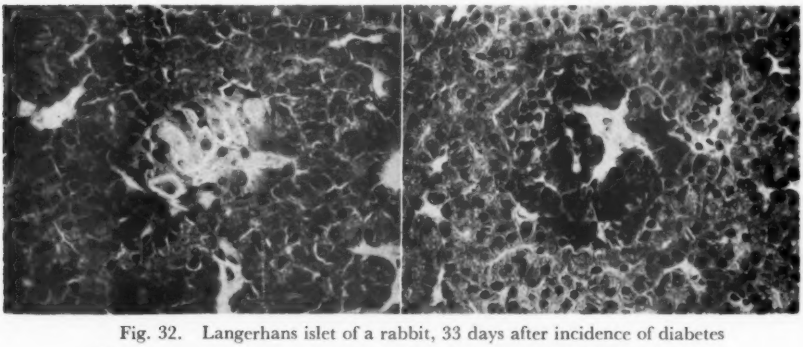

Fig. 32. Langerhans islet of a rabbit, 33 days after incidence of diabetes with intravenous injection of nicotine (Gomori's staining). Marked hydropic degeneration in β-cells, similar to those in fig. 31, but the degeneration here is somewhat more advanced.

Fig. 33. Langerhans islet of a rabbit, 33 days after incidence of diabetes with intravenous injection of nicotine (Gomori's staining). Islet almost made up of α-cells only, a few markedly degranulated β-cells being observable among them. A large gap, seemingly formed by necrosis and collapse of β-cells visible in a part.

Fig. 34.

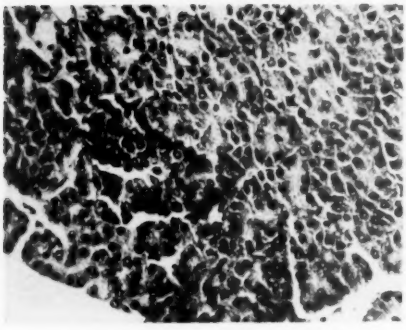

Fig. 34. Langerhans islet of a rabbit, 387 days after incidence of diabetes with intravenous injection of nicotine (Gomori's staining). Islet almost entirely composed of atrophied α-cells only.

Fig. 36. Fig. 37.

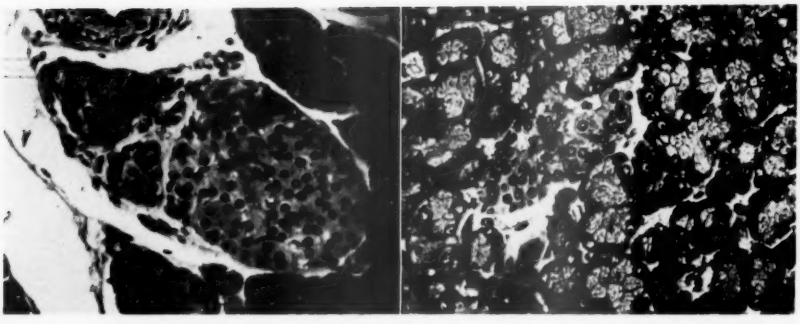

Fig. 36. Langerhans islet of a normal rat (Gomori's staining). β-cells constitute the chief component, α-cells situate mostly in the periphery.
α-cells · · · dark (red) β-cells · · · light (blue)

Fig. 37. Control. Langerhans islet of a rat 10 days after intravenous injection of 50 mg./kg. of alloxan (Gomori's staining). Urine sugar ++++ during observation and blood sugar 522 mg./dl. at time of death.

Only a small portion of β-cells are seen surviving, the remainder being taken up by the unaffected α-cells, so that the islet appears as sorely shriveled up.

Fig. 38. Fig. 39.

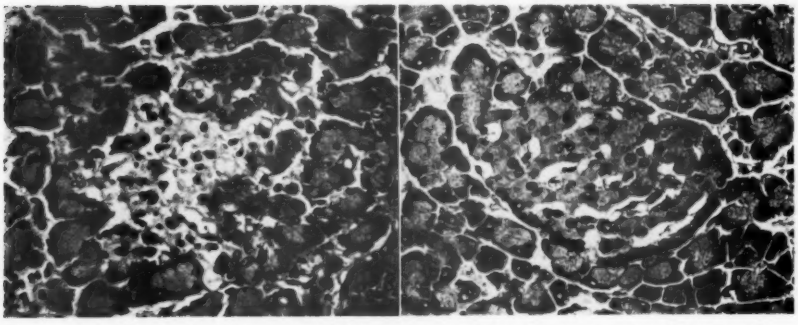

Fig. 38. Control. Langerhans islet of a rat 5 days after intravenous injection of 50 mg./kg. of alloxan (Gomori's staining).

Urine sugar ++++ during observation and blood sugar 410 mg./dl. at time of death.

β-cells are mostly decomposed and nearly absorbed off, the remaining few showing nuclear pycnosis. Around these some unaffected α-cells are found in the periphery.

Fig. 39. Langerhans islet of a rat injected with 25 mg./kg. of benzimidazole, 5 min. later with 50 mg./kg. of alloxan, both intravenously, and sacrificed 10 days afterwards (Gomori's staining).

Urine sugar −∼± during observation and blood sugar 112 mg./dl. at time of death.

Most of β-cells are seen unchanged, and also α-cells unchanged.

Fig. 40. Fig. 41.

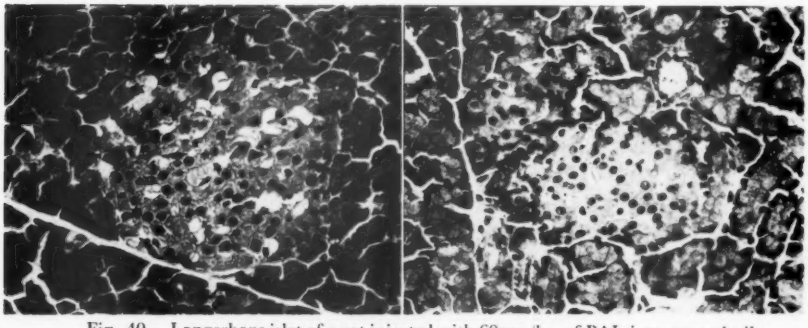

Fig. 40. Langerhans islet of a rat injected with 60mg./kg. of BAL intramuscularily, 1 hour later with 50 mg./kg. of alloxan, and sacrificed 10 days afterwards (Gomori's staining). Urine sugar −∼± during observation and blood sugar 128 mg./dl. at time of death.

Pycnosis of nuclei is observed in a few of the β-cells, but most of them and α-cells are seen unchanged.

Fig. 41. Langerhans islet of a rat injected with 200 mg./kg. of sodium bicarbonate, 5 min. later with 50 mg./kg. of alloxan, both intravenously, and sacrificed 5 days afterwards (Gomori's staining).

Urine sugar −∼± during observation and blood sugar 103 mg./dl. at time of death.

Pycnosis and deformation of nuclei are observed in a few of the β-cells, but most of them and α-cells are seen unchanged.

Fig. 42. Fig. 43.

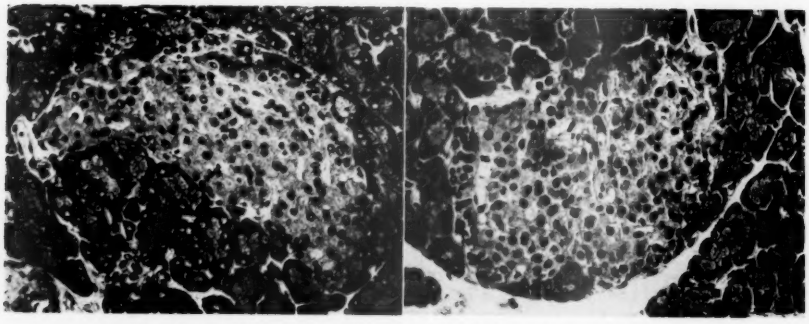

Fig. 42. Langerhans islet of a rat injected with 200 mg./kg. of thiochrome, 5 min. later with 50 mg./kg. of alloxan, both intravenously, and sacrificed 10 days afterwards (Gomori's staining).

Urine sugar $-\sim\pm$ during observation and blood sugar 88 mg./dl. at time of death.

β-cells are seen nearly unchanged and also α-cells unchanged.

Fig. 43. Langerhans islet of a rat injected with 0.75 mg./kg. of vitamin B_{12}, 5min. later with 50 mg./kg. of alloxan, both intravenously, and sacrificed 10 days afterwards (Gomori's staining).

Urine sugar $-\sim\pm$ during observation and blood sugar 113 mg./dl. at time of death.

In a few of the β-cells, pycnosis and deformation of nuclei and decomposition of protoplasm are observed, but most of them and α-cells are seen unchanged.

Fig. 44. Fig. 45.

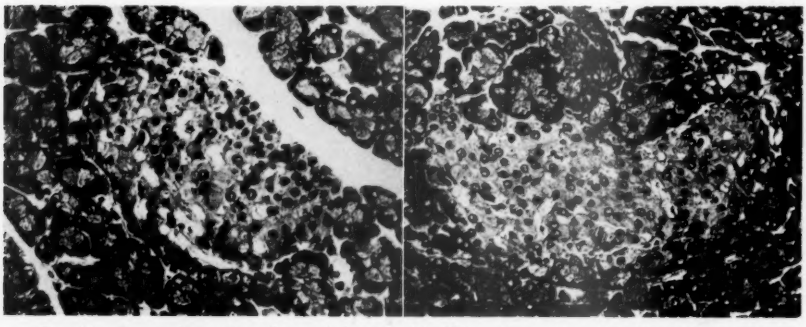

Fig. 44. Langerhans islet of a rat injected with 200 mg./kg. of anthranilic acid, 5 min. later with 50 mg./kg. of alloxan, both intravenously, and sacrificed 10 days afterwards (Gomori's staining).

Urine sugar $-\sim\pm$ during observation and blood sugar 131 mg./dl. at time of death.

In a few of the β-cells, pycnosis and deformation of nuclei and reticular and vesicular decay in protoplasm are observed, but most of them are seen unchanged, and in α-cells no change.

Fig. 45. Langerhans islet of a rat injected with 100 mg./kg. of ursodesoxycholic acid, 5 min. later with 50 mg./kg. of alloxan, both intravenously, and sacrificed 10 days afterwards (Gomori's staining).

Urine sugar $-$ during observation and blood sugar 102 mg./dl. at time of death.

β-cells are seen nearly unchanged and also α-cells unchanged.

Fig. 46. Fig. 47.

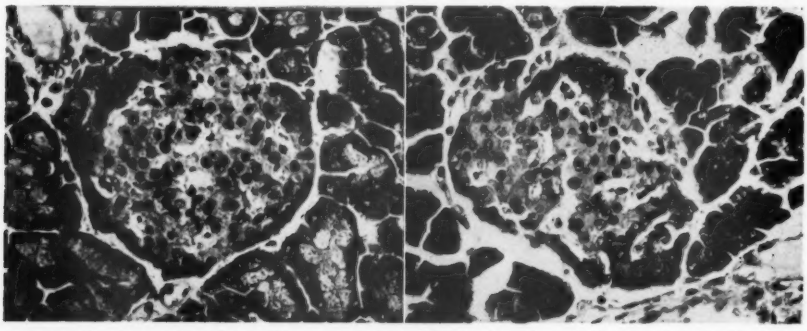

Fig. 46. Langerhans islet of a rat injected with 180 mg./kg. of theobromine, 5 min. later with 50 mg./kg. of alloxan, both intravenously, and sacrificed 10 days afterwards (Gomori's staining).

Urine sugar — during observation and blood sugar 112 mg./dl. at time of death.

In a few of the β-cells, pycnosis and decomposition or vesicular delay of protoplasm are observed, but most of them and α-cells are seen unchanged.

Fig. 47. Langerhans islet of a rat injected with 500 mg./kg. of glutathione, 5 min. later with 50 mg./kg. of alloxan, both intravenously, and sacrificed 10 days afterwards (Gomori's staining).

Urine sugar $-\sim\pm$ during observation and blood sugar 103 mg./dl. at time of death.

Little change in β-cells (in a few of them, pycnosis of nuclei and decomposition of protoplasm are seen) and none in α-cells.

Fig. 48. Fig. 49.

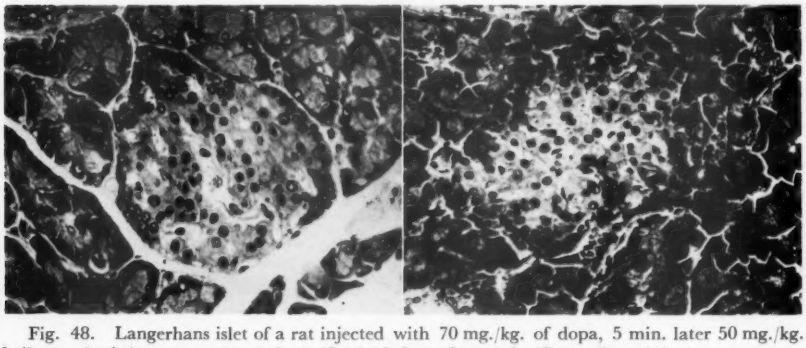

Fig. 48. Langerhans islet of a rat injected with 70 mg./kg. of dopa, 5 min. later 50 mg./kg. of alloxan, both intravenously, and sacrificed 10 days afterwards (Gomori's staining).

Urine sugar — during observation and blood sugar 95 mg./dl. at time of death.

In a few of the β-cells, pycnosis of nuclei and decomposition of protoplasm are observed, and no change in α-cells.

Fig. 49. Langerhans islet of a rat injected with 100 mg./kg. of L-cystine, 5 min. later 50 mg./kg. of alloxan, both intravenously, and sacrificed 10 days afterwards (Gomori's staining).

Urine sugar $-\sim\pm$ during observation and blood sugar 141 mg./dl. at time of death.

In a few of the β-cells, pycnosis and deformation of nuclei and decomposition of protoplasm are observed, but most of them and α-cells are seen unchanged.

Fig. 50.

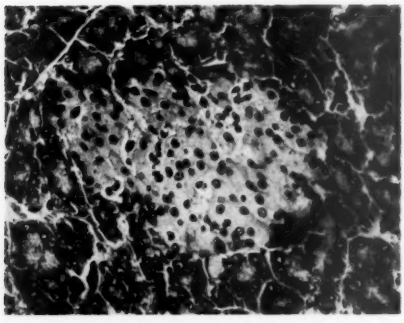

Fig. 50. Langerhans islet of a rat injected with 500 mg./kg. of DL--creatinine, 5 min. later 50 mg./kg. of alloxan, both intravenously, and sacrificed 10 days afterwards (Gomori's staining).

Urine sugar $-\sim\pm$ during observation and blood sugar 92 mg./dl. at time of death.

Little change in β-cells (in a few of them, pycnosis or decomposition of nuclei are seen) and none in α-cells.

Fig. 51. Fig. 52.

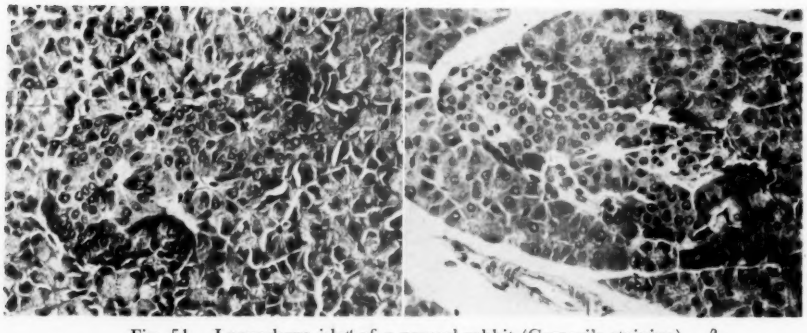

Fig. 51. Langerhans islet of a normal rabbit (Gomori's staining). β--cells chiefly composing the islet. α-cells situated mostly in the periphery, a few also observed more centrally.

α-cells · · · dark (red) β-cells · · · light (blue)

Fig. 52. Langerhans islet of a F_4 rabbit No. 11433 not yet diabetic (Gomori's staining). Sacrificed 120 days after birth. The islet is rather large and consists mainly of β-cells, with α-cells forming the periphery. About a dozen of the β-cells show nuclear pycnosis, but no other degeneration is noted, though the β-cells are much, and the α-cells slightly smaller than in normal rabbits.

Fig. 53.

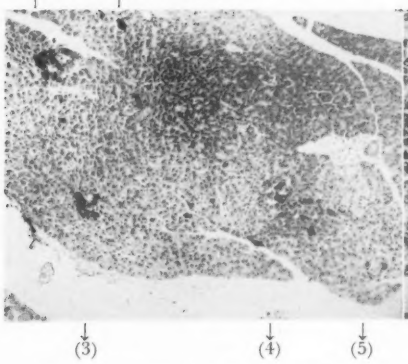

Fig. 54.

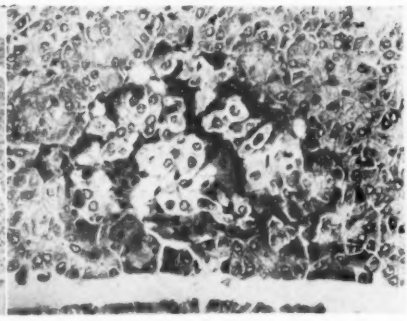

Fig. 53. Langerhans islet in the same rabbit as Fig. 2. Same staining. Low magnification. 5 islets seen. The islets in low left (3) and right center (4) consist almost only of α-cells. The large islet in upper left (1) is also mainly made of *a*-cells. The oblong islet next on the right of it (2) and that in the extreme right (5) consist mainly of β-cells. The β-cells in all the 5 islets are much, and the *a*-cells, a little, reduced in size.

Fig. 54. Langerhans islet of a F_4 rabbit No. 11424 with spontaneous diabetes (Gomori's staining). Severe diabetes, with glycosuria persisting for 10 days. Died 146 days after birth. Swollen and markedly hydropic β-cells and strongly atrophied β-cells with dark-staining protoplasm and strong nuclear pycnosis are seen. α-cells with no marked change scattered in the periphery.

Fig. 55.

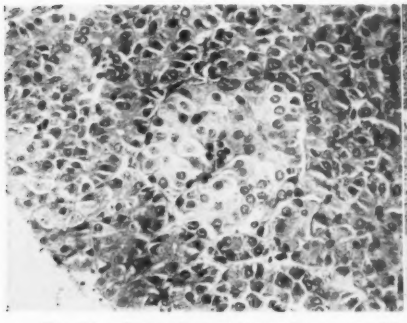

Fig. 56.

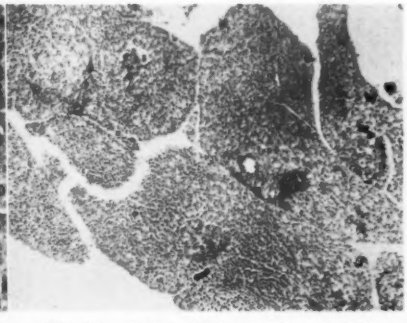

Fig. 55. Langerhans islet in the same rabbit as Fig. 54. Same staining. Islet consisting almost only of swollen β-cells showing marked hydropic degeneration.

Fig. 56. Langerhans islet of a F_4 rabbit No. 11427 with spnotaneous diabetes (Gomori's staining). Severe diabetes, with glycosuria persisting for 41 days. Died 151 days after birth. Low magnification. 5 islets in sight, all mainly composed of β-cells, but none of them normal, affected with manifold degeneration. Most of the β-cells in the comparatively light-looking islet in the extreme left show decreased granules. The islet next to the right of the above show severe atrophy and nuclear pycnosis (highly magnified in Fig. 58). The islet in the low center also show the same degeneration, as well as that in the extreme right. In the islet at the right center, two spherical, blood-filled, dilatations of blood vessel are seen. The β-cells here show sometimes nuclear pycnosis, atrophy or hydropic degeneration.

Fig. 57. Fig. 58.

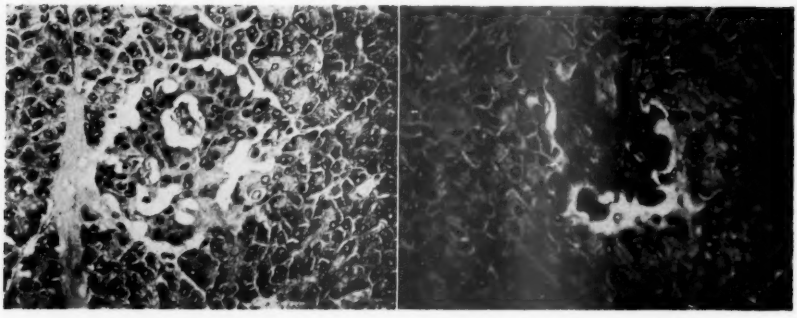

Fig. 57. Langerhans islet in the same rabbit as Fig. 56. Same staining. In some peripheral and inner parts, figure of collapsed β-cells and gaps of eliminated β-cell debris are observed. The remaining β-cells are almost all strongly atrophied, showing nuclear pycnosis, degranulation, and seemingly on the verge of dissolution. A very small number of them show hydropic degeneration.

Fig. 58. Langerhans islet in the same rabbit as Fig. 56. Same staining. The β-cells are severely atrophied, their nuclei pycnotized, their protoplasm dark-violet stained and their boundaries indistinct.

Fig. 59. Fig. 60.

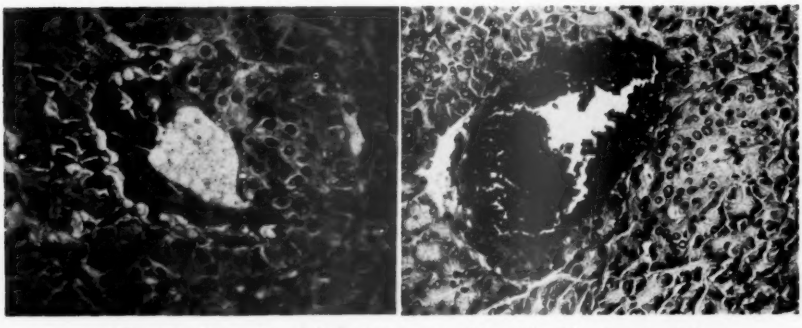

Fig. 59. Langerhans islet in the same rabbit as Fig. 56. Same staining. A large interstice at the center, containing a little cotton-like substance. Most of the β-cells in the right part of the interstice show hydropic degeneration while those in the left and lower part are mostly atrophied and pycnotized.

Fig. 60. Langerhans islet in the same rabbit as Fig. 56. Same staining. The islet almost taken up by marked dilated blood vessel, the cells around them severely atrophied. The few visible α-cells are not markedly changed.

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